

How to regain lost leadership

The United States is making heavy weather of recasting its distant plans for the exploration of space, chiefly because it seeks leadership without asking what is meant by leadership.

A fractured leg may prevent a person from walking about, but the frustration it engenders can also be a powerful stimulant of ambitious plans for future expeditions. This seems to be the spirit in which the United States, with the space shuttle still grounded, is asking itself insistently where it will go next. Mars? Back to the Moon? Or somewhere else? There is nothing reprehensible about the cheerful confidence in the future which these questions imply; the shuttle will indeed be flying again one day, probably even more successfully than in the two years preceding the accident at Cape Canaveral eighteen months ago. Then there will be the space station to build, enough to keep people busy for much of the 1990s. Public and much private imagination in the United States centres on what will happen after that. The down-to-earth report to NASA (National Aeronautics and Space Administration) from Ms Sally Ride, the astronaut, which has now been issued (see *Nature* **328**, 284; 1987), should help to ground fevered speculation in substance.

In practical terms, the recommendations seem derived from the motto "Do not try to run before you can walk!" Specifically, the report concludes that the expedition to Mars, for which there has recently been a groundswell of imperfectly-informed support, is not an option in its own right, but is instead dependent on the development of technology and techniques for doing less spectacular things reliably. To get to Mars safely, people will have to practise getting to the Moon. To do either, as well as to prepare for an uncertain future, there will have to be more deliberate attempts to stimulate new technology. Meanwhile, there is a great deal to be learned about the Solar System from a rounded programme of observation such as that which the planetary science community has been urging on NASA for several years. Ride, who is off to Stanford soon, will find that her report has won her friends in her new workplace.

But why do any of these things? And will any of them assuage the yearning to regain "leadership" in space sharpened by the Challenger disaster? The Ride report takes up this question only obliquely, providing again a list of choices of what meaning can be read into the concept of leadership in much the spirit that a Congregational minister will offer his congregations a choice between alternative concepts of the deity. Ride's caution is not merely forgivable but understandable. While some small part of the \$10,000 million a year that NASA spends may be justifiable on utilitarian grounds (telecommunications and so on), and some other small part is comparable with what governments spend on basic research of different kinds, most of this considerable sum is aimed at less tangible goals whose choice is ultimately political, and on which the US administration and the Congress will eventually decide. (The Ride report, as predicted, says they might with advantage have done so sooner.) The obvious drawback is that the government seems no better placed than the man in the street to chart a course for the more distant future.

Muddle about leadership has confused US policy on space since the beginning. It was a good wheeze, thirty years ago, that the US Navy thought of using its familiarity with military rocket technology to launch a civilian satellite (Vanguard) as part of the US contribution to the International Geophysical Year; the fact

that the Soviet Sputnik came along a few months earlier had the United States worrying about leadership even before it had launched a rocket. In this meaning of the word, leadership means "prowess", the capacity to excel at everything. The Apollo project to send people to the Moon was directly inspired by the spurious sense of failure engendered by Sputnik but, against the odds, succeeded brilliantly both as a piece of exploration and in restoring falsely-injured pride. So why not abandon that dull work, and build a shuttle craft instead? That is how the logic ran just over a decade ago, which explains where the United States is now.

It is important that, even if the shuttle Challenger had not blown up in January 1986, those in the United States with an anxious frame of mind would still have been able to worry about lost leadership. There would have been the Hubble telescope (now waiting on the ground), it is true, but just look at that long list of record-breaking Soviet endurance flights! In retrospect, can it even have been wise to leave close encounters with Halley's comet to Europe and the Soviet Union? Can it be safe to let the French (who invented *son et lumière*) make the running in space-based illuminations? What is the danger that some space power so far undeclared will embark on a project not yet thought of — quarrying the heat at the surface of Venus as a source of energy or simply putting a national flag around the planet? The trouble with these haunting speculations about what others may do is that their sheer variety is distracting. The quest for leadership through prowess is a recipe for doing a great many things, but none of them particularly well.

That, implicitly, is what the Ride report says. A decision now to send an expedition to Mars might satisfy the immediate wish to be seen to be doing something out of the ordinary, but would only add to the widespread sense of leadership lost if something should go wrong. Not that such a consequence is unavoidable. Indeed, the paradox in the depressed reaction of the United States to the Challenger accident is that there appear to be so many people who believe it shameful that there should be accidents even in projects as technically advanced as the shuttle. Leadership of this kind appears to equate with infallibility.

A wiser view is that leadership is not won by technological pyrotechnics (or by spending money), but must be earned. The United States space programme has done well in that respect over several decades. It has been open from the start, while the speed with which bright ideas have been turned into usable machines (telecommunications satellites again) has been an international public service. So too, in due course, will be the Hubble telescope. Where the programme has failed is in its tendency not to build on modest successes and even breath-taking successes. Some of those who turned up their noses at the Apollo programme may have regretted the retreat from the Moon. The humdrum exploration of the Solar System (without the help of people), while still substantial, has too often been skimped when the budget process has been rough. Yet those dull tasks may in the long run be better ways of earning respect abroad, and better ways of creating the depth of technical competence that will be needed for the long haul, than attempts to second guess the competition and outdo it. □



Super Collider brings 25 states into contention

- Illinois and Texas seen as heavyweights
- Competition is chance for flag-waving

Washington

"A GOOD clean fight — so far" is how one participant described the contest to win the Superconducting Super Collider (SSC) location. The object of the competition, for which 43 sites in 25 states were proposed to the Department of Energy (DoE) by the deadline of 2 p.m. on 2 September, is a 52-mile circumference, 20 TeV proton accelerator, but the real prize is a multibillion-dollar facility with thousands of immediate jobs and the potential to attract high-technology industry and a desirable population to the chosen area.

The deadline had been postponed for one month after Congress altered the rules to disallow direct financial incentives from the states as a factor in the selection. Even so, the true cost, dependent especially on labour rates and the price of electricity, may vary considerably from site to site. A committee under the aegis of the National Academies of Sciences and Engineering will ponder cost, technical suitability, availability of communications and quality of life to reduce the entries to a shortlist of six, from which the DoE will select the favoured site. Only then will any state-provided incentives (cheap electricity, tunnelling at no cost, housing at reduced rates and so on) be revealed; these bonuses are attached to the bids in sealed envelopes, whose contents some states have been advertising with gusto.

Thirty-four of the sites have the official backing of their states (New York is offering four locations, and six other states have two options). The remaining nine bids come from a variety of local government and business consortia; five of them are in Texas, where the state investigated 14 sites before settling on two. DoE has said that sites must be entirely on US territory, ruling out one of the New York proposals, which crosses into Canada, as well as the site described as "Moon Area L-5", submitted by one Paul Jablonka.

California, regarded as a strong contender, was almost a non-starter when official backing was held up until a policy on the use of minority contractors during construction was agreed. The proposal, for two sites in the Sacramento area, reached Washington in time, but vocal opposition from local farmers and environmental groups, as well as legislative wrangling, may have tainted the bid enough for DoE to become shy of it.

The two biggest players seem to be

Texas and Illinois. In addition to a site near Dallas-Fort Worth, which officials say comes out better than any site in the nation for its combination of cost, geology and quality of life, Texas has also put forward a location near Amarillo, in the western part of the state, whose appeal is its rock-bottom price. Texas may have earned itself a small black mark last year, when the Texas Accelerator Center (TAC) protested that the SSC Central Design Group had unfairly dismissed its magnet from the SSC design process. Peter McIntyre of TAC hopes that the scientific community is "mature enough to forget" the dispute.

The Illinois application is unique: it sees the SSC as an add-on to the existing facility at Fermilab, whose present main ring could serve, with small modifications, as the injector for the new device. This is presented as an advantage both in cost and in the presence of a ready-made pool of talent and ability.

Opposition to Illinois arises mostly from sympathy for the underdog, but with a particular twist. There is a theory that Fermilab was built in the midwest at least partly to assuage critics who complained that the east and west coasts were monopolizing research. Now Fermilab, grown up into the scientific establishment, is on the other side of the same argument.

One way of avoiding a controversial choice between the major contenders would be to go for a dark-horse candidate, among which North Carolina and Colorado are often mentioned. North Carolina has a site near its well-established Research Triangle, which boasts several biotechnology companies and three universities; Colorado has the Aspen Center for Physics and the National Center for Atmospheric Research, as well as the attractions of the Rockies, near its Denver location.

A number of states honestly assess their chance of winning the SSC as small. Oregon, for example, can offer cheap hydroelectricity but has no real high-technology base. But these candidates regard the money spent on making a bid as an investment. Their SSC proposals are easily adaptable into advertisements for the state's attractions to industries in general, and can also be used to persuade state governments to improve amenities. But a handful of states have spent so much money that not getting the SSC will be an embarrassment.

David Lindley

Lab worker infected with AIDS virus

Washington

AN epidemiological study at several centres of individuals working with human immunodeficiency virus (HIV), the virus causing AIDS (acquired immune deficiency syndrome), has identified a worker who became infected through handling the virus.

HIV antibodies were first detected in the exposed individual approximately a year ago. But it has now been shown that the virus isolated from the infected individual — whose identity has not been revealed — is indistinguishable from the one the worker was handling.

The study, headed by William Blattner of the National Cancer Institute (NCI) and Stanley Weiss, until recently also at NCI, examined serum from more than 200 individuals at 15 different facilities in six states. The facilities were chosen because of their routine use of HIV in research. The infected individual worked at a facility where very highly concentrated quantities of HIV were being produced.

Safety investigators involved in the case from the outset are still looking into possible modes of transmission. A statement released by NCI says the worker in question had no other known risk factors for HIV. Among the possibilities being considered is that the individual may not have been wearing all standard protective garments, despite working in a special facility designed for the highest level of containment of hazardous viruses.

The concentration of virus found in the laboratory in question is "unlike the concentration found anywhere outside a laboratory", according to the NCI statement. Robert Gallo, head of NCI's laboratory of tumour cell biology and one of Blattner's collaborators, says the person was probably working with concentrations of virus higher than most AIDS researchers.

Gallo is nevertheless concerned that the incident may be blown up out of proportion, causing unnecessary concern among laboratory workers. The Centers for Disease Control have recently issued revised recommendations for safety procedures for health care workers occupationally exposed to the AIDS virus. NCI plans to reexamine its procedures for people working with concentrated virus.

Restriction analysis, performed by Gallo's laboratory, showed that the virus isolated from the infected individual was indistinguishable from the virus with which he or she was working. This is apparently the first documented case of a researcher infected with HIV through occupational exposure.

Joseph Palca

Japanese budget requests for international collaboration

Tokyo

SUPERCONDUCTORS, AIDS (acquired immune deficiency syndrome), the Human Frontiers Science Program and sequencing of the human genome feature in the budget requests for fiscal year 1988 submitted by Japanese government ministries and agencies last week. But tough negotiations lie ahead with the Ministry of Finance over the budget proposals.

The budget requests total ¥60.8 million (\$430,000 million), 12 per cent up on this year. Sales of shares of Nippon Telephone and Telegraph, which will net the government several million million yen, will be used to cover part of the increase and to refund government bonds (about 20 per cent of the budget is swallowed by debt-servicing payments). Nevertheless, the Ministry of Finance plans to slash the requests to around ¥56 million million by the end of this year when the budget will be submitted for Diet approval.

At the end of August, the Council of Science and Technology, Japan's top science policy-making body which is Chaired by Prime Minister Yasuhiro Nakasone, recommended promotion of research into the immune, cerebral and nervous systems over the next ten years, attaching particular importance to AIDS research. The Health and Welfare Ministry has requested more than ¥2,000 million (\$15 million) to combat AIDS in fiscal 1988. Although still small the budget request is more than an order of magnitude larger than this year. More than half (¥1,265 million) is set aside for the development of anti-AIDS drugs and vaccines, including animal experiments. And \$1.5 million is requested for the World Health Organization's anti-AIDS programme.

The council also calls for basic research into the new superconducting oxides discovered by IBM Zurich last year.

The Science and Technology Agency

hopes to get ¥2,362 million (\$17 million) in fiscal 1988 plus a similar amount (¥2,839 million) in credit for the next two or three years to support superconductor research (the former budget is allotted to specific institutes, whereas the latter credit account is open to application from the agency's institutes). Over ¥2,500 million (¥1,035 million in fiscal 1988 and ¥1,511 million in three-year credit) is assigned to the National Research Institute for Metals to build high-field magnets (80, 40 and 20 Tesla) which will be used to characterize the high-field properties of the new ceramics. The National Research Institute for Inorganic Materials also gets a large portion (¥280 million in fiscal 1988 and ¥931 million in two-year credit) for a new high-powered electron microscope to analyse the atomic and molecular structure of ceramic crystals.

MITI has requested comparable funding in the superconductor field. More than ¥1,500 million (\$10 million) goes to basic theoretical work on the new high-temperature superconductors (¥770 million) and their processing into thin films and devices (¥850 million), largely under the ministry's category "basic technology for future industries" which includes research on biochips. An even larger sum (¥1,670 million) is assigned to development of a 70,000-kW superconducting generator, in MITI's energy-saving 'Moonlight Project'; conventional superconducting wire will be used in the armature of the generator. MITI plans to establish a private non-profit foundation, the "international superconductor industry and technology promotion centre", to support research on the new ceramics (see *Nature* 328, 282; 1987).

MITI's Eletrotechnical Laboratory has established links with the US National Bureau of Standards and the ministry's budget request includes ¥20 million for joint research with the United States on superconductors. A programme to invite foreign researchers to Japan will also be created under the Science and Technology Agency's special promotion fund.

The prime minister asserted at the meeting of the Council of Science and Technology that the Human Frontiers Science Program, Japan's ambitious plan to promote international research into biological functions, will go ahead. But the funds requested for fiscal 1988 are small compared with the scale of project originally envisaged by MITI (¥1 million million over 20 years).

MITI officials point out that, although the Frontiers budget is still small, several closely related programmes may eventually come under the wing of Frontiers. The Science and Technology Agency has requested ¥247 million for research and development of techniques for sequencing long DNA sequences, such as the human genome.

David Swinbanks

Shuttle booster fired (at last)

Washington

AFTER irksome delays caused first by a broken water hose and then (twice) by failure to reset all computers to a new countdown (*Nature* 329, 3; 1987), the redesigned booster for the space shuttle was successfully fired on 30 August at Morton Thiokol's Wasatch Facility in Utah. It ran at full thrust and for the full two-minute duration of the shuttle boost phase. Engineers will take several weeks to dismantle and inspect the booster and to analyse the data from 520 instruments measuring pressures, temperatures, strains and so on, but officials at both Morton Thiokol and NASA were evidently pleased by the belated but eventually trouble-free firing.

The purpose of this first full-scale test was to prove the basic soundness of the redesigned joints between the cylindrical sections of the booster body. A third O-ring has been added, along with a metal lip to clasp the sections together. The failure of an O-ring in subfreezing temperatures caused the loss of Challenger last year, but

after much searching Morton Thiokol engineers found no replacement material with better overall properties. Instead, electric heaters have been installed in the O-ring mountings.

During the redesign, engineers also took the opportunity to change the construction of the nozzle, especially its attachment to the body of the rocket. This had always been regarded as a potential weak spot in the booster, long before the Challenger explosion.

Depending on the success or otherwise of three tests scheduled for November this year and February and March next year, as many as three firings of production-model boosters will be conducted between April and June 1988.

There has been some criticism that no test of a single isolated booster can truly simulate launch conditions, but short of an unmanned shuttle launch there is little else that can be done. Engineers at Morton Thiokol, their morale revived after last year's slump, say the tests will be "as good as they can be".

David Lindley



Indian turmoil over planned US tests of new vaccines

New Delhi

A ROUGH passage is predicted for the trials in India of genetically engineered vaccines proposed under the agreement between the United States and the Indian Department of Biotechnology (DBT) signed in July (see *Nature* 328, 287; 1987). Apart from the ethics of using Indians for trying out new vaccines, questions have been raised about the desirability of giving the United States access to militarily sensitive data on epidemiology and immunity profiles of the population.

DBT insists that there is no basis for concern and that the agreement was signed only after interministerial consultation and cabinet approval. But Dr Pushpa Bhargava, director of the Centre for Cellular and Molecular Biology at Hyderabad, says that "such an important agreement" should not have been signed without consideration by DBT's own scientific advisory committee, of which he is a member.

In a public statement, Bhargava warns against trials with imported genetic vaccine before "we are in a position to design and do every trial and test ourselves". He also complains that the government has done nothing to set up research and production facilities in biotechnology, but is willing to "depend on what we receive from outside".

Sharing Bhargava's views, Dr D. Banerjee, chairman of the Centre of Social Medicine and Community Health of the Jawaharlal Nehru University in New Delhi, describes the agreement as "most disturbing".

In another development, an official of the National Institute of Communicable Diseases (NICD) under the Health Ministry has made it clear that NICD is opposed to open trial of the rabies-vaccinia recombinant virus vaccine for controlling rabies in stray dogs. This is one of the several vaccines the United States had offered for trials.

The controversy has been further sharpened by provisions in the agreement allowing the participation of private US companies in the trials, and US representatives on the environmental risk-assessment committee. In addition, India cannot enter into contracts or hire professional services without US approval.

Access to epidemiological data is another issue. The United States planned, as part of the programme, an epidemiology centre to develop a computerized database on "health-profile and demographic information" of well-defined populations. That such information is needed to assess a vaccine's efficacy is not denied, but whether it should be shared

with an outside agency is questioned.

According to Bhargava, epidemiological data on immunocompetence "can be of strategic importance". The absence in India of yellow fever, or of a single case of overt AIDS (acquired immune deficiency syndrome), may be due to a peculiar immunocompetence of the Indian population; those knowledgeable about developments in biological warfare resent any collaboration that might enable outsiders to study the immunological "herd structure" that apparently provides a natural barrier against certain infections.

DBT says that the idea of an epidemiology centre has been shelved and that the \$1.2 million earmarked in the agreement is for epidemiology research and training. Countering allegations that Indians will become guinea-pigs, DBT says that no vaccine will be tried without "prior clearance of the ethics committee of the Indian Council of Medical Research and the Drug Controller of India".

According to DBT, the programme aims chiefly at developing new vaccines against the major diseases of India through joint research with US scientists. DBT also says that the controversy is premature because specific details of the projects have yet to be worked out, and that they will have to be cleared internally before being presented to the US side.

One of the internal bodies, whose clearance is mandatory, is the high-level committee set up in 1975 by the cabinet specifically to screen all foreign-aided research projects from the "security and sensitivity angle". It is headed by the cabinet secretary and includes the scientific adviser to the Defence Ministry, the secretary to the Department of Science and Technology and the other administrative secretaries whose portfolios are affected.

Among other things, the committee must satisfy itself that the project is under Indian control and that there is no military implication. Under the committee's guidelines, "the study should not allow access by foreigners to sensitive information or data", and projects involving communicable diseases, biological and virological studies must be particularly subjected to "searching and critical scrutiny".

Because of the controversy already created by the agreement, the committee is likely to examine DBT's proposals thoroughly before granting clearance. Some say the controversy could have been avoided if the United States had been content with training Indian scientists in vaccine technology, leaving the clinical trials entirely to the Indians.

K.S. Jayaraman

Israelis scrap Lavi

Tel Aviv

THE Israeli government announced last week that it has decided to scrap the controversial Lavi fighter jet project, causing thousands of Israel Aircraft Industries (IAI) workers to react with fury and threaten to "cut off the state of Israel". But their nationwide protests — blocking runways at Ben-Gurion Airport, setting up roadblocks on major highways and manhandling officials — were spurred less by support for Lavi than by fear that dreams of "lifetime employment" are now gone.

The treasury and defence ministry are now frantically trying to minimize the number of dismissals at IAI and its subcontractors. An estimated 4,000 workers could lose their jobs over the next few years, many of whom may leave Israel if alternative projects are not quickly found. At the beginning of this week, some 15,000 protesters vowed to bring down the government unless a way was found to revive Lavi.

L.P.

Seabed mining for India

New Delhi

FOLLOWING the formal acceptance and registration of its claim by the executive body of the preparatory commission of the International Seabed Authority in New York last month, India is now entitled to mine manganese nodules from an area of 150,000 km² of the seabed of the Indian Ocean, about 3,000 km south of the tip of the Indian land mass.

K.S.J.

RAWMAC reservations

London

THE British government's independent Radioactive Waste Management Advisory Committee has again expressed reservations about the ability of existing facilities to cope with increasing amounts of low-level nuclear waste. The committee's new chairman, Professor John Knill, says there is a risk that the 300-acre low-level dump at Drigg, in Cumbria, "will be put under pressure" following the government's decision last May to abandon plans to find a new low-level waste site, which would have been operating by the early 1990s (see *Nature* 327, 93; 1987). Furthermore, estimates for the total volume of waste expected during the next 25 years have been revised upwards by 30 per cent to 500,000 m³, mainly due to the removal of contaminated surface soil at British Nuclear Fuel Ltd's new thermal oxide reprocessing plant, under construction at Sellafield.

Knill says the abandonment of the shallow-site investigations have resulted in the loss of geological data that could have been of value for investigations into a deep-disposal site for low- and intermediate-level waste.

S.H.

ANZAAS north of Capricorn looks to its neighbours

Townsville, Queensland

THIS year's meeting of ANZAAS (Australian and New Zealand Association for the Advancement of Science) at the end of August was no worse for being roughly half as big as in previous years. Moreover, the meeting successfully broke with the diffuse pattern of previous years, concentrating instead on the theme of science and life in the tropics.

Unlike previous ANZAAS meetings, this year's also reflected the character of its host university — James Cook University, Australia's only university north of the Tropic of Capricorn with a modest (by Australian standards) student body of 4,000 and only 300 academics. Since its foundation in 1965, James Cook has been conscious of its responsibility (and of the political need) to interact (and to be seen to interact) with its regional community, not merely the third of Australia within the tropics but also Australia's northern neighbours.

This explains why most of the papers read at the meeting dealt with tropical research — tropical rain forests, cyclones, the exotic dangerous animals and venoms of northern Australia, aboriginal health and welfare, women living in isolation and the anthropology of this tropical region.

James Cook's regional interests are beginning to yield benefits. Professor Ray Golding, the chemist who is the university's vice-chancellor for this year, says that he had no difficulty in raising A\$4 million from the travel and tourism industry to found Australia's first chair in tourism. The university is also committed to establishing a centre for tropical health and medicine and an institute for tropical rain-forest studies. (The long-established Centre for Tropical Marine Studies

already has an international reputation.)

As always, ANZAAS attracted a larger throng of reporters than any other science meeting in Australia, whence one of this year's controversies — the wide coverage given to the personal assertions of Dr Robert Endean, a zoologist at the University of Queensland (Brisbane) that the infestation of the Great Barrier Reef by the Crown of Thorns starfish (*Acanthaster planci*) is the result of increased fishing for the natural predators of the starfish, the giant triton and the groper.

Although Endean has been publicizing his human-cause theory for the past 20 years, it was eagerly taken up as new. By comparison, the contradictory evidence presented at the meeting, much of it original, that these infestations are not new, having recurred in cycles for at least the past 2,000 years, was conveyed to the wider public as if it were a conservative and institutionally defensive response to an environmental threat.

This illustrates one of the still-recurring faults of ANZAAS. Because speakers are not required to produce a full paper in advance, and because the proceedings are not published, there is no opportunity for proper peer-criticism of the theories put forward at individual meetings. In the event, when challenged here, Endean admitted that the evidence for his theory is only circumstantial, but the pressure remains on politicians to provide funds for the eradication of starfish from the Great Barrier Reef.

True to form for ANZAAS, there was no meaningful discussion at this year's meeting of the condition of Australian science, made even more perilous by the *de facto* demotion of Mr Barry Jones, the minister for science. Peter Pockley

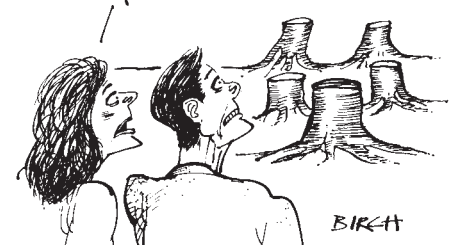
Test without EPA approval has come to an end

Washington

AN experiment involving environmental release of genetically modified microorganisms that began without US Environmental Protection Agency (EPA) approval came to an end last week. Gary Strobel, a Montana State University researcher, cut down and burned the elm trees he had injected with a modified strain of bacteria to protect them against Dutch elm disease. He said he ended the experiment because it might be a stain on the university's reputation, and he did not want to jeopardize his colleagues' work.

The EPA has issued "mild sanctions" against Strobel for flouting the regulations governing the field-testing of modified microorganisms. Strobel failed to get EPA approval before injecting 14 trees on campus in June with a strain of *Pseudomonas syringae* that he had modified to enhance its ability to produce an antibiotic that wards off Dutch elm disease. EPA regulations require researchers to seek approval from the agency 90 days before

Pity he couldn't protect them from Paranoia...



carrying out an environmental release of modified microorganisms. But waiting for EPA approval would have meant postponing the experiment to next year's growing season, so Strobel began injecting trees several days after he mailed his application to the EPA. For the next year, EPA sanctions require Strobel to have a "responsible party" cosponsor any field tests of recombinant organisms. In addition, he will have to seek approval from the university's biosafety committee.

Strobel's actions have raised doubts within the scientific community. Many feel that his actions were unwarranted because he sidestepped the peer-review process that forms the backbone of science. Others are concerned that the widespread publicity of the case endangers the nascent "goodwill" toward biotechnology held by the general public, and weakens the public's faith in the government's ability to regulate new technologies. But the federal rules governing biotechnology are reckoned to be complex and disjointed, weaknesses highlighted by Strobel's defiance. Carol Ezzell

Indian nuclear power goes public

New Delhi

NUCLEAR power generation is to go public with the passing of a bill in the Indian parliament. The new company, Nuclear Power Corporation of India Limited (NPCIL), will be responsible for the design, construction and operation of atomic plants. These functions are at present carried out by the Nuclear Power Board, a wing of the Department of Atomic Energy, which will be abolished.

According to Mr K.R.Narayanan, the Science Minister, the main reason for setting up the company is shortage of government funds to support the proposed expansion of the nuclear power programme. The plan is to increase nuclear power capacity from the present

1,230 MW to 10,000 MW by the year 2000. The estimated cost is more than \$10,000 million, which obviously cannot come from budgetary allocations. The NPCIL will raise the money from banks, financial institutions and by floating tax-exempt bonds in the capital markets. It is expected that the company will become financially self-reliant by 1995 and not require budgetary support after that.

The NPCIL will work closely with state electricity boards, which will purchase the nuclear electricity, and will depend on the Bhabha Atomic Research Centre for research and development. The anti-nuclear lobby hopes for greater accountability from NPCIL.

K.S.Jayaraman

Money problems at NERC hit marine science laboratories

London

A FINANCIAL crisis alternately described as a "short-term cash-flow problem" or "the brink of bankruptcy" has hit the UK Natural Environment Research Council (NERC). Marine science laboratories are facing a serious funding shortfall, and research vessels remain tied up because sudden budget cutbacks mean there is no money for fuel. Some NERC community research programmes have been halted for the remainder of the financial year.

The marine division is especially feeling the pinch, after the Institute of Oceanographic Studies (IOS) lost a £2-million government contract to study deep disposal of high-level radioactive waste. The research had been going on for several years, but was unexpectedly stopped, amid accusations of a lack of government planning for future waste disposal.

Marine science laboratories throughout Britain are all being asked to tighten their belts. In addition to the IOS, programmes have also been pared down at the Marine Biological Association (MBA)'s laboratories at Plymouth, the Scottish MBA, NERC's Institute for Marine Environmental Research at Plymouth, the Proud-

man Oceanographic Laboratory at Bidston, and the Sea Mammal Research Unit at Cambridge.

NERC's funding squeeze is being blamed partly on an increase in staff salaries and partly on a shortfall in the government's science vote, which provides 70 per cent of NERC's £100 million budget.

Although the research council says its community programmes of directed research are its first priority, several of these interdisciplinary projects, including the Joint Global Ocean Flux Study and a North Sea study, have been frozen until the new financial year.

NERC is trying to put into practice the government's intention that more research should be funded by industry and other outside sources. Two seminars have been scheduled for late in the year, designed to promote more interest in commissioning research work.

Cost savings are a stated goal of the proposed merger between the two Plymouth-based marine laboratories, the Marine Biological Association and NERC's Institute of Marine Environmental Research. The creation of a joint facility was first proposed by a House of

Lords select committee, and later supported by a NERC visiting group. But NERC has been accused of trying to push the proposal through in a hurry.

"You might expect that with all the funding problems we're facing, 1987 is not a very opportune time for a major change like this", says MBA's acting director, Dr Eric Corner, who complains of "a very tight time schedule". Although negotiations are still proceeding, NERC says the new merged laboratory facility should be approved later this month and operating by next April.

MBA's council and membership still have to consider the merger, and some staff are not happy with the new prospects. But with nearly 95 per cent of the laboratory's funding provided by NERC, there is a feeling that cooperation with the plan is obligatory.

Kathy Johnston

Radiation limits challenged

London

THE International Commission on Radiological Protection (ICRP) is being publicly challenged by 800 scientists who are asking for tighter radiation standards, in the light of revisions on the assessment of radiation doses received by Hiroshima and Nagasaki atomic blast survivors.

The ICRP is reviewing its ten-year-old recommended radiation-exposure limits during its two-week meeting now under way in Como, Italy. Members are considering new data on cancer rates in Japanese atomic bomb survivors, after US epidemiologist Edward Radford reported that radiation doses were lower than originally estimated because of the screening effect of buildings. Existing dose limits are partly based on risk calculations derived from studies of the Japanese survivors.

Signatories to the petition say that new calculations show that risk estimates for fatal cancers from exposure to radiation are between two and five times too low. The ICRP is being asked to reduce five-fold the radiation dose limits for nuclear and other radiation workers, now set at 50 millisieverts per year, and to pay more attention to risks of non-fatal cancers.

The weighting factor used for exposure of gonads underestimates the risk of genetic damage and ignores the cancer risk, the critics say, and they also point out the sensitivity of fetuses to radiation.

Signatories to the petition include Nobel laureates Linus Pauling and George Wald, former Manhattan project scientist Joseph Rotblat, and Karl Morgan, a former senior member of the ICRP.

No decisions on changes to the radiation limits are expected from the ICRP before next autumn after more discussions.

Kathy Johnston

Paris science museum under attack

Paris

FRANCE'S controversial science and technology museum at La Villette in north-east Paris has come under attack from Jacques Valade, minister for research and technology. In an interview on French television last week, Valade said that the museum is "too expensive", as a result of "its youth and of the inadequacy and imprecision of initial budget estimates".

The state has provided FF600 million (£60 million) per year since the museum was opened 18 months ago, representing 80 per cent of its running costs. The museum itself yields about FF75 million while the 'Géode' — a geodesic dome housing a panoramic cinema — provides a further FF50 million in revenue.

On the basis of a confidential report by the inspector-general of finances, Mr Pierre Consigny, Valade said the year's results from La Villette were "terrifying", with 59 per cent of the models out of order. This figure has been contested by Mr Maurice Lévy, who retired as director of the museum on 7 September. Lévy puts the figure at 15–20 per cent and says that the failure rate is due to a number of factors: an exceptionally high percentage of interactive models, their heavy usage, the fact that many are prototypes and an inadequate maintenance budget.

The museum was conceived under Valéry Giscard d'Estaing's government, to be built instead of new abattoirs at La Villette, a project which created a scandal when it was abandoned in 1971, having cost FF950 million. The plan to build a science museum was adopted by the social-



The Géode at La Villette.

ists, under Mitterrand, and was inherited by Chirac on coming to power last year.

In answer to Valade's call for better financial management, Lévy said that 30 per cent of running costs could be provided from revenue, so long as the number of visitors continues to increase and given a more aggressive marketing strategy. Lévy envisages hiring space in the museum to private high-technology companies, the production and sale of audio-visual materials and the manufacture of 'executive toys'. A conference centre, recently opened on the site, is booked until July 1988 and the Géode typically sells 95 per cent of its seats.

Peter Coles

Franco-US nuclear services agreement

Paris

FACED with a world-wide slump in the market for nuclear technologies, three leading French companies, Framatome, Cogéma and Uranium Péciney, have teamed up with Babcock and Wilson, the second largest manufacturer of reactors in North America. To be called B & W Fuels Inc., the new company will be the first Franco-American company in the nuclear sector. It will sell nuclear fuels made at Lynchburg in Virginia and offer expertise in reactor-core maintenance to US electricity utilities, competing with Westinghouse, which services about 70 per cent of the US market.

Babcock and Wilson, which built the ill-fated reactor at Three Mile Island, hope that the unblemished safety record of the French companies will boost its credibility. At the same time, the link-up reflects a general restructuring of the nuclear industry, with increasing international collaboration. Framatome, Cogéma and Uranium Péciney already collaborate in France, and together offer experience of the whole nuclear fuels cycle.

France, the second largest nuclear nation, has been looking for an opportunity to penetrate the coveted US market but has always come up against tight protectionist barriers. As Babcock and Wilson will be majority shareholders of the new company, with 51 per cent of the stock, these obstacles should disappear. Because of a world slowing in demand for new reactors, the French hope to sell their expertise in the 'service' industries of core maintenance, uranium extraction, enrichment and reprocessing.

The United States abandoned reprocessing irradiated waste in 1976, in favour of storage, both as a result of a drop in uranium prices and for fear of creating a proliferation of plutonium. Reprocessing is worthwhile only for nations having a nuclear energy output exceeding 25,000 MW. Of these nations, France is possibly the world leader in reprocessing technology, the others being West Germany, Britain and Japan.

Cogéma, a subsidiary of the French atomic energy commission (CEA), processes about 80 per cent of irradiated fuels from all light-water reactors and is shortly to open a new plant in La Hague, bringing its capacity to 1,600 tonnes per year by 1992. The potential demand from clients is considerable. Unless the United States starts reprocessing again (which it pioneered), it could be faced with an estimated 40,000 tonnes of irradiated waste by the year 2000.

Peter Coles

UK rush on superconductivity centre widely criticized

London

HURRIED moves by the Science and Engineering Research Council (SERC) to set up Britain's first University Research Centre in high-temperature superconductivity (see *Nature* 328, 370; 1987), have provoked widespread unease within the academic community. Serious doubts are being expressed about the wisdom of choosing such a fast-moving, unpredictable field of research for the first centre, and many observers are convinced that SERC's haste is a direct result of pressure from central government in a misguided effort to counter international competition.

In July, 11 institutions were invited to submit bids to host the centre, which will concentrate primarily on small devices. There has been almost universal condemnation of the lack of time for the institutions to formulate their proposals — with a deadline of 15 September, and during a period when many academics are either on vacation or attending overseas conferences. SERC hopes to announce the location of the centre before the end of the year, and to see it running by next summer.

The most commonly expressed fear is that the time and effort needed to establish the centre will mean less time spent in the laboratory. Furthermore, the declared aim of giving industry a hand in the funding and management of the centre (the

first of several, in various subjects, to be established — see *Nature* 328, 464; 1987) could, according to some, detract from the more basic research.

Of the institutions invited to bid for this first centre, Imperial College London and the Universities of Bristol and Leeds will not submit a proposal. The Universities of Birmingham and Warwick, which already collaborate in this field, are thought to be preparing a joint application. The Universities of Southampton, Cambridge, Oxford and Strathclyde will submit individual proposals. The University of Liverpool is to put forward a proposal for a 'north-west consortium' involving Liverpool Polytechnic, the Universities of Keele and Lancaster, SERC's Daresbury Laboratory and several industrial partners, including ICI's advanced materials laboratory in nearby Runcorn. Strathclyde University will be seeking to emphasize its strong industrial links. The University of Durham is looking to team up with the University of Newcastle on Tyne and, possibly, Newcastle Polytechnic, as well as various industrial organizations.

The sizes of the bids will vary considerably, depending mainly on whether the institutions are asking for new buildings. One bid is thought to be asking for around £7 million in capital, another around £4 million.

Simon Hadlington

Calm waters for Hughes Medical Institute

Washington

MANY troubled months for the world's largest scientific charity have come to an end with the election of a new president for the Howard Hughes Medical Institute.

Last week, the institute announced that Purnell W. Choppin, vice-president since 1985, is taking over as president. He fills the post left vacant in June when Donald Fredrickson, former director of the National Institutes of Health, resigned after a bitter dispute with the institute's trustees. The institute has not made the details public

but it appears that both Fredrickson's policies and the involvement of his wife in the institute had caused controversy.

Choppin, formerly a professor of virology at the Rockefeller University, has no wish to speak of the past as the institute enters a phase of rapid growth. Next year, the number of faculty-level investigators will go up from 163 to 186, and researchers and support staff from 1,170 to 1,600 at the institute's 27 laboratories.

The institute has so far operated by building well-financed units, much envied by the ordinary researcher, within leading laboratories. That policy will change next year with an increasing emphasis on appointment of individuals outside the large units. "Resources will be spread to a much larger number of institutions and to support good scientists where we find them", says Choppin. The institute's settlement with the Internal Revenue Service, which enabled it to maintain its status as a medical research organization (see *Nature* 326, 236; 1987), permits moves into new project areas, provided sufficient funds are spent directly on medical research.

Alun Anderson



Purnell Choppin takes over.

Irish make R&D a cross-border cement

Dublin

Cross-border science and technology will benefit to the tune of IR£7.2 million from the International Fund for Ireland (IFI), the IR£60-million fund set up by the governments of the United States, Canada and New Zealand after the signing of the Anglo-Irish agreement in 1985. The objective of the fund is to encourage industrial and economic development in the six counties of Northern Ireland and the six bordering counties of the Republic of Ireland, although the board of the fund is also prepared to back projects on an all-Ireland basis.

The provisional allocation from the fund to science and technology is reasonably generous. Investment and business will get IR£19 million, agriculture and fisheries IR£4 million. The board is insisting that grants will be repaid out of royalties and other earnings.

Under its science and technology programme, the board has already approved a grant of IR£100,000 to the Queen's University, Belfast, for the provision of technical services to industry and a grant of IR£250,000 to the Magee campus of the University of Ulster (the amalgam of the University of Ulster at Coleraine and the Belfast Polytechnic which came into being two years ago) to found a development chair in information technology.

IFI has also agreed to support for a first planning and evaluation year a centre for environmental processing and analytical chemistry at Queen's University, Belfast. The idea is that the centre will raise funds from industry by inviting companies to become associate members.

A still more ambitious scheme, to which IFI is prepared to commit IR£2 million, is for an all-Ireland centre of technological excellence, possibly concentrating on software, microelectronics or new industrial materials. This will link together institutions north and south of the border, between which the funds will be divided. Queen's (Belfast), Trinity College (Dublin) and University College (Cork) are in the running, but the project is at an early stage, with firm proposals invited.

The programme in science and technology is being administered jointly by the National Board for Science and Technology of the republic and the Department for Economic Development of Northern Ireland. After the present fund has been allocated, the board expects to issue a call for supplementary funds later in the year, but expects that all its money will have been allocated by early in 1988.

Mary Mulvihill

US under pressure to harpoon Japan's whaling plans

Washington

JAPAN and the United States may soon be on a collision course over Japan's insistence on continuing its whaling operations in defiance of International Whaling Commission (IWC) recommendations.

The World Wildlife Fund (WWF) last week called on President Ronald Reagan to impose "strict economic sanctions" against Japan's fishing industry if it sends out its whaling fleet to the Antarctic in October. A major campaign to win the support of Congress and the public is following. The president is empowered by the Pell and Packwood Amendments to ban fish imports and to cut catch quotas in US waters of any country that "diminishes the effectiveness" of the IWC. Either move would hit Japan hard.

Mount Fuji rumbles

Tokyo

JAPAN'S Mount Fuji is rumbling. And the Japanese Meteorological Agency has rushed a seismograph to the volcano's summit to monitor the quakes which began at the end of last month. But there seems to be little danger of eruption.

Mount Fuji, which lies above the subducting Philippine plate where it slices into the mainland of Japan, last erupted in 1707. Explosions occurred about halfway up the south-east flank of the volcano and spewed out about a cubic kilometre of volcanic rock and ash, some of which reached the site of present-day Tokyo, about 100 km from the volcano. But since that time Fuji has been dormant.

On 20 August, personnel in the meteorological agency's observatory perched on the rim of the crater at the summit felt the ground tremble, and by 1 September there had been nine minor earthquakes (magnitude less than two on the Richter scale). With the climbing season on Mount Fuji in full swing and with the eruption of nearby Mount Mihara on Oshima island still fresh in the minds of agency officials (see *Nature* 324, 805; 1986), the agency hurriedly installed a portable seismometer. And scientists now want a permanent summit station.

But researchers at Tokyo University's Earthquake Research Institute, which maintains seismographs on the flanks of the volcano, scoff at the publicity surrounding the event. They point out that about ten similar minor quakes occur every month. Moreover, the sharp P-wave arrivals of the recent earthquakes suggest they are of tectonic rather than volcanic origin, according to Professor Yoshiaki Ida of the institute. Harry Glicken

In 1986, the United States came close to applying sanctions when Japan defied an IWC ruling to stop commercial whaling from 1986 to 1990. But the dispute ended in a compromise agreement by which Japan agreed to stop commercial whaling from 1988. Now the WWF accuses Japan of breaking the terms of the agreement.

Japan argues that it will stop commercial whaling in 1988 as agreed. But it is going to substitute "scientific whaling" which is allowed by an international agreement permitting the killing of whales for scientific research. Japan's "scientific" research plans, which involve killing 875 minke whales and 50 sperm whales per year, have already been castigated by IWC's scientific committee as unlikely to add any useful knowledge. And as the whales will be caught by the same boats, and their meat sold on the commercial market, "scientific" whaling looks very like commercial whaling in disguise.

WWF research scientist Roger Payne is particularly concerned over plans to kill sperm whales. The 50 whales to be killed would be males, which are much larger and thus of greater commercial value than females. The number of sperm whale males has already been reduced to levels where reproduction of the species is endangered, he believes. Minke whales may be under less of a threat, but nobody has sure knowledge of safe catch levels. Data on population biology is sorely needed but, according to WWF president William Reilly, cannot come "from the study of dead whales".

Iceland has also just announced plans for scientific whaling and will be put under pressure by WWF calls for sanctions. But Japan remains the most important target as it controls most of the world market for whale meat.

WWF will try to sway Japanese opinion by collecting one million signatures on a petition to the Japanese government "respectfully requesting" that the moratorium be observed. If the petition is successful, commercial whaling may end for the foreseeable future, well beyond the end of the moratorium in 1990. Once whaling facilities have been closed, it is unlikely that it will be economic to re-establish them. The Soviet Union, which took the largest catch of whales after Japan, has already decided to abandon whaling.

Whether the US administration will take action in time to halt the departure of Japan's whaling fleet for the coming Antarctic summer remains to be seen. Any sanctions will have to be considered in the light of the many problems besetting US-Japan trade relations. Alun Anderson

South Africa row overshadows Mainz archaeology congress

Mainz, West Germany

ONE year overdue, the embattled eleventh congress of the International Union of Prehistoric and Protohistoric Sciences (IUPPS) finally took place here last week, amid anti-apartheid protests and a boycott by some African and European researchers.

The rift within the archaeology and anthropology communities over apartheid in South Africa has, if anything, deepened since September last year, when the rival World Archaeological Congress was held in Southampton, England.

The row over South African participation began in September 1985, when the organizers of the Southampton congress succumbed to pressure from several quarters and decided to ban South African and Namibian scientists from the 1986 Southampton congress (see *Nature* 317, 754; 1985). The IUPPS, which had previously been the sponsor of the quinquennial meeting, told the Southampton organizers that the meeting had to be open to all, or it would risk losing IUPPS sanction. The organizers were unable in the end either to appease the IUPPS or reinvite the South Africans. The IUPPS Permanent Council decided in January 1986 to remove its blessing from the Southampton congress (see *Nature* 319, 524; 1986) and began planning a separate congress in Mainz.

The decision of the Southampton organizers to go ahead with their meeting,

which came to be called the World Archaeological Congress (WAC), stemmed in part from their belief that archaeology requires the participation of researchers outside the industrialized West, which is why members of aboriginal groups such as eskimos and Native Americans, as well as scientists from many developing countries, were invited to the WAC. It was the participation of so many researchers from less-developed countries that brought the South Africa issue to the fore. A movement to boycott the Mainz meeting unless changes were made in the IUPPS constitution grew out of the Southampton meeting.

Despite statements by the organizers



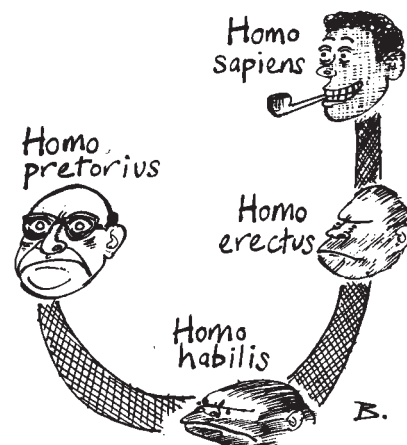
J. Desmond Clark and Phillip Tobias

here that participants are attending as individuals and not as members of national delegations, the presence of South Africans at both the congress and the IUPPS Permanent Council meetings has been enough to trigger a student protest and calls for a boycott. More significant is the withdrawal of several African countries such as Nigeria, Uganda and Angola.

The Mainz congress nevertheless drew 864 registered participants (as of 4 September), most of whom opposed any sort of boycott. There was a noticeable dearth of British researchers, according to John Yellen of the US National Science Foundation, but other developed nations, East and West, were amply represented. The relatively sparse turnout of US researchers was attributed by several people to scheduling difficulties. Others added that the substance of the Mainz meeting would not have been particularly interesting for most US archaeologists.

The high point of the meeting seems to have been the discussion on the prehistory of Africa held on 2 September, not least because of the paper by Phillip Tobias (Department of Anatomy, University of the Witwatersrand) on the origins of human speech. His conclusion, based on endocasts made from some early hominid fossils, that even *Homo habilis* had some sort of language capability, provoked vigorous discussion, especially among some younger participants with recent field experience.

Another successful session on Africa was led by J. Desmond Clark of the Uni-



versity of California at Berkeley, who, together with Tobias, gave one of the two lectures that opened the Mainz conference. Clark's lecture dealt, among other things, with the question of how early man may have crossed from Africa into Europe — whether by land across the isthmus of Suez or by a variety of water routes. The land route seems the more likely, but Clark asserted that a water crossing would have been possible even at that early stage.

Congress organizer Konrad Weidemann admits that sections on the neolithic period and some other areas were weak, primarily because "half of the Romanians didn't show up". But he attributed their absence to financial rather than political reasons.

Virtually no Africans have come to Mainz. Congress organizer Konrad Weidemann of the Römisch-Germanisches Zentralmuseum in Mainz said this was entirely due to lack of finances, a statement disputed by advocates of a boycott.

The IUPPS plans to return to its five-year schedule with the 1991 congress, which will probably be held in Bratislava, Czechoslovakia. The Czech invitation, said Weidemann, includes the understanding that scientists of all nationalities, including South Africans, will be guaranteed access.

Some participants were nevertheless "lobbying hard" for changes in the IUPPS that would prevent the weakening of ties between European and African scientists. Amini Mturi of the Department of Antiquities of Tanzania is trying to get the IUPPS to implement three recommendations to ease the situation: an end to South African representation on the Permanent Council; an IUPPS condemnation of apartheid; and a review of the IUPPS statutes, which are generally acknowledged to be out of date. If Mturi fails in his attempt to influence the IUPPS to implement these changes, he said, "this might be my last congress". Furthermore, if Mturi fails, or even if he succeeds, Tanzania might ban any archaeologist who has worked in South Africa.

Steven Dickman

ICSU to Beijing

THE International Council of Scientific Unions (ICSU) has moved the venue of its next general assembly, planned for next year in Japan, from Tokyo to Beijing, chiefly because of the requirements of the Japanese government of intending South African participants at the meeting. The decision was taken at the June executive committee meeting of ICSU, which learned that there had been an insufficient response to a request that South African participants should not be required to sign a declaration of their opposition to apartheid while applying for Japanese visas. This provision does not apply to others from South Africa wishing to visit Japan — businessmen, for example — and apparently derives from the terms of a UN resolution (accepted by Japan) in favour of a boycott of South African sport and "culture". It is believed that the Science and Technology Agency of China will be able to assure free access to Beijing next year, although formal word to this effect has not been received. □

Science the only hope?

SIR—In his commentary, Erwin Chargaff (*Nature* 327, 199; 1987) voices his objections to certain experiments in the field of human reproduction and to the commercialization of the making of babies. He knows that his objections will have little or no impact, and his moving last paragraph reflects his own state of hopelessness. I recommend all students of the technology of human reproduction to read his article, though I do not share its desperation.

Raised in the same winter of decaying culture in Austria, Chargaff and I have known each other for many decades. We have always agreed to disagree on virtually every aspect of human endeavour, particularly science. I object to his pessimism; he objects to my optimism.

In his article, Chargaff proclaims the end of an era in which science was the never-ending search for truth about nature — a quest that would help us in understanding the workings of our world. With the splitting of the atom and the gene, this era, he says, was displaced by a new one of manipulation and deflection of the forces of nature. Once again I disagree. With all the naiveté I can muster, I believe that science is still the only hope for *Homo sapiens*.

I am an optimist, as defined by a nuclear physicist, as a person who believes that the future of *Homo sapiens* is uncertain. I do not believe that political and economic solutions alone will solve our problems. I believe that we have a chance of decent survival only if we can learn about the roots of aggression, hate and greed — if we can learn to raise a new generation that will sublimate the aggressions that once upon a time were the forces that led to the evolution of our society, such as it is. I contest Chargaff's pronouncement of a new era of manipulation of natural forces. It is not new, and it will not and should not displace our search for truth. We have always manipulated the forces of nature — we built houses with lightning rods; we eliminated microorganisms that caused epidemics; we killed animals that were enemies or suitable for food consumption; we erected prisons for criminals. Why is it unethical to manipulate a diseased gene? Is it unethical to learn how to repair it just because nature has created it? Is this not another form of creationism, of accepting nature's verdicts without protest?

I am fully aware of the grave ethical questions that we must face, but ethics comes in at the level of execution and not at the level of search. The discovery of the splitting of the atom was not evil, but the use of this knowledge for the destruction of this world is, although if we let it happen, maybe we deserve it.

It is criminal for a childless couple to get a child by killing its natural parents

(*Nature* 327, 552; 1987), but is it wrong to give a child to a mother by manipulating the fertilization process?

Granted, we do not know, as Chargaff points out, whether such an unnaturally implanted egg or a frozen embryo will breed the same product as its natural sibling. Probably not, and we should tell this to the prospective parents. But how can we tell without trying whether there is a risk? Is it not even possible that an ovum surviving the clumsy hands of the surgeon will be better equipped than its natural equivalent?

Chargaff is distressed by the monetary gains involved in the technology of human reproduction. So am I, but is this relevant to the basic problem? Among the failings of *Homo sapiens* is greed, a form of aggression that we cannot control. Greed may be unethical or even criminal when it leads to theft or unnecessary surgery; it is a fact of life and not an argument against advances in human reproduction.

I am not a white knight riding on a high horse. I shall not even know whether I am riding a horse or an ass, until, as the Chinese say, the dust settles. I also do not know why we search for truth, and I like to probe into the biological origins of this drive. Yet I believe that it is among the best gifts nature has given us, a gift that may eventually get us out of the mess Chargaff rightly bemoans.

EFRAIM RACKER

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Vive la différence

SIR—The apparent innuendo by your reviewer Alan Nunn May (*Nature* 327, 377; 1987), that the United States of President Truman needed deterring as much as the Soviet Union of Comrade Stalin, cannot be allowed to pass unchallenged.

If we assume that the United States and the Soviet Union were equivalent in this respect, then it must follow either that the United States and the Soviet Union were equally (un)democratic or that democracy makes no difference.

Both propositions have, I suppose, been advanced by apologists for those whose penetrating intelligence and concern for humanity led them to choose to work for Stalin. I accept neither: the United States is democratic; the Soviet Union is not, and never has been. And democracy does make a difference.

J.F. CRAWFORD

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Vatican and IVF

SIR—The Vatican condemnation of *in vitro* fertilization (*Nature* 326, 229 & 268; 1987) seems to stem mainly from the elimination of surplus fertilized eggs that this method inevitably entails.

It is of course the right of churches to express ethical views about reproduction as about any other aspect of human life. But to be considered seriously when dealing with a physiological activity, these views should take account of biological constraints. In this context, it has not been stressed that sexual reproduction, in all species, is characterized by an immense destruction of gametes, embryos and juveniles, which may at first sight appear a reckless waste. But all biologists know this is essential for the maintenance of the genetic quality of the species. Even in humans, a slowly reproducing animal with few young, fewer than a score of the 500-odd ova produced by the female are ever likely to develop into advanced pregnancy. Taking into account infant and child mortality, the frequency of sterile couples and the death of young mothers linked with delivery, it can be calculated that, in many primitive societies, the average number of children per fertile woman must have neared 8 (in less than 20 years) just to produce the two adults necessary to maintain the population.

In advanced societies, hygiene and modern medicine have progressively reduced the losses of fetuses, infants and children. The equilibrium could only be maintained by the introduction of contraceptive techniques which means the elimination of earlier stages in the reproductive process. Incidentally, the balance is not easy to achieve. Some European peoples now have too few children. On the other hand, when the impact of modern hygiene has been too rapid on psychologically unprepared societies, there is a population explosion.

Anyway, when this destruction of potential human life takes place as early as possible, it leads to a reduction of the total amount of suffering. As long as the Church does not recognize the biological necessity of this elimination, its views can be dismissed. Now, whether fertilization occurs in the "natural" way or is medically assisted, seems morally irrelevant. Is renal dialysis, bypassing the physiological way of making urine, to be damned because it is not "natural"? This does not deny the Church the right to have strong views about other, not strictly biological, aspects of the problem such as surrogate mothers and the choice of sperm donors.

HENRI FIRKET

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The great ozone controversy

While new data on the Antarctic springtime ozone hole does not bear directly on the problem of ozone destruction worldwide, it does significantly shift the balance of belief.

GENERAL impatience with the process of science will be moderately enhanced by the important article on ozone in the Antarctic atmosphere by C.B. Farmer and his colleagues on page 126 of this issue. Farmer's group has made the first accurate measurements of the presence of simple halogen compounds in the springtime ozone hole in the Antarctic stratosphere, yet go no further than to say that their observations are "entirely consistent" with the hypothesis that there is a cause and effect relationship between the presence of the halogens and the absence of ozone. Even J.C. Farman and J.A. Pyle, writing less formally, go no further than to say that they believe the connection is causal, but in language suggesting that dissenters are free to believe differently.

Why, when professionals cannot make up their minds even about what is happening in the Antarctic, should the world's diplomats be locked in negotiation of the fine print of the Vienna Convention on the Preservation of the Ozone Layer? That is what a great many outsiders (not to mention some interested parties) will be asking. Do chlorofluorohydrocarbons deplete the atmosphere of ozone, or do they not? And if not, why all the fuss?

Yet circumstances like these are common. It is now more than thirty years since the demonstration (using British data) by Bradford-Hill and Doll of a strong correlation between cigarette-smoking and the occurrence of lung cancer, but a large part of that interval has been occupied with arguments about the likelihood that the connection could be causal.

Indeed, the passage of time does suggest that the relationship may be more complicated than it seemed at first. There may be genetic factors. It could be that cigarettes are blackened in the eyes of potential users by environmental influences not recognized at the outset (the previously unsuspected high concentration of radon in some parts of the world, for example). Yet what survives through thirty years of argument is that the correlation is so strong, and its consequences statistically so steady, that doubts about the reality of the correlation are melting away.

So shall we have to wait for thirty years before knowing what these chemicals do to ozone in the stratosphere, and find then that we all have skin cancer? Should not

problems of cause and effect be simpler in the physical sciences, where it is possible to measure things with arbitrary precision, where the variations between the entities observed are not as great as they are among people and where the causal links are derived from immutables such as Newton's laws?

The simple riposte is that there are many people working in the observational physical sciences who wish that were indeed the case. Ask any physical oceanographer or meteorologist.

Ozone measurements have been plagued with difficulty since they were first begun by Dobson, at Oxford, in the 1930s. For much of the interval, there have been instrumental difficulties, only satisfactorily resolved in the past ten years with the development of sensitive solid-state infrared detectors for recording the flux at the ground (or elsewhere) of solar radiation at the absorption frequency of ozone.

Then there are problems of interpretation. How to distinguish between the ozone layer in the lower stratosphere and ozone in the troposphere? Are fluctuations in time, on a timescale of half an hour or so, a sign that stratospheric ozone is patchy or of some temporary imbalance in the competing processes of formation and destruction at high altitudes? Does the elevation of the line of sight to the Sun matter?

At various times, all these effects have been offered as explanations of puzzling measurements. Ironically, whatever may be the effect of manmade chemicals on the ozone in the lower stratosphere, it is probable that activities nearer the surface of the Earth, by increasing the amount of low-level ozone, have complicated the problem of using Dobson instruments to tell the secular trend of ozone in the atmosphere. In principle, satellite instruments using the ultraviolet reflectance of the atmosphere should avoid many of these difficulties by giving a more synoptic measurement, but their sensitivity is not yet as great.

Farmer's measurements are in some ways more easily interpreted. The springtime decline of ozone in Antarctica was first recognized from historical records from Halley Bay (J.C. Farman, B. G. Gardiner & J.D. Shanklin, *Nature* **315**, 207; 1985) and on the basis of measurements in the spring of 1984 (September

and October). Since then, there have been only two Antarctic springs (the third is about to begin).

Farmer and his colleagues (also based at Halley Bay) were lucky last year to find themselves alternatively outside and inside the polar vortex that seems to keep a column of polar atmosphere as an almost isolated air mass for months on end. What they find is that there are high concentrations of simple halogen molecules in the Antarctic atmosphere, which may not surprise those who have always believed that halogenated hydrocarbons are the death of ozone, but that the properties of the column within the polar vortex differ surprisingly from expectation.

With hindsight, it is easy to believe that the cold atmosphere above the winter pole would be a physical sink for halogenated hydrocarbons such as those used in aerosol cans and refrigerators. Pyle and Farman raise the interesting question of the form in which these materials are condensed before being made gaseous, and destructive of ozone, by the Sun's return. Others will raise the possibility that the winter poles may be more permanent sinks for them, places from which they are removed from the low winter stratosphere by precipitation, and will go on collecting snow for analysis from the Antarctic.

Meanwhile, the grounds for believing that halogenated hydrocarbons destroy ozone are changed in only one respect, important though that may be. The striking correlation between low concentration of ozone and high concentrations of halogen compounds in one exceptional part of the atmosphere, coupled with the expectation from laboratory results that the former should be destroyed by the latter, is the equivalent in this connection of a demonstration in biology that a process observed *in vitro* occurs *in vivo* as well. So it is natural to believe that the ozone hole has been explained. That is what Farman and Pyle say.

But nobody, for the time being, has direct evidence that the same materials destroy ozone globally. If the winter pole takes halogenated hydrocarbons permanently out of circulation, it might thus ameliorate what happens elsewhere. But that this behaviour might compensate for the annual discharge of chemicals to the atmosphere is against the odds. The diplomats had better keep talking.

John Maddox

Protein crystallography

Catching up with fast changes

Dagmar Ringe

DESPITE its limitations, the technique of millisecond protein crystallography promises to extend the range of biochemical problems that can be studied by X-ray diffraction. The work of J. Hajdu *et al.*, reported on page 178 of this issue, demonstrates the potential of using the static tool of crystallography to gain insight into the workings of a protein as it changes conformation during a reaction.

Biochemists have spent much time and energy on the elucidation of enzymatic mechanisms, devising clever methods to identify the reactive groups (those essential to the catalytic process) in a protein. Even cleverer methods can identify intermediates along the reaction pathway. Anomalous results — unexpected spectral changes, for instance — are ascribed to conformational change, the universal catch-all. The protein is attributed with flexibility, some of it caused directly by the chemical process being catalysed.

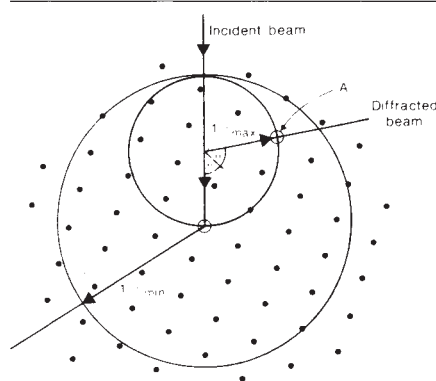
The advent of protein crystallography, allowing structures and changes in structures to be observed directly, promised a radical enlightenment. Unfortunately, partly because of a misunderstanding of the limitations of crystallography, the hoped-for information has not turned out to be as helpful as was expected. The long time periods (days) required for the collection of protein crystal-diffraction data means that the structures obtained are positional averages, and any short-lived intermediates or structural motions — for example, conformational changes — cannot be observed. Structural information is therefore limited to species that are stable for long times, such as enzyme-product complexes when product inhibition is strong, and any structures that occur in between are lost. Attempts to trap intermediates by, for example, the use of low temperatures, have met with only limited success.

A new approach to this problem recently appeared in the form of molecular engineering. Carefully selected changes in the identity of reactive groups in an enzyme could, in principle, alter the mechanism so as to trap intermediates or provide chemical evidence for the function of a specific group in the mechanism. But careful structural analysis of mutants designed to elucidate specific functional questions either has not been done or has failed to explain the change in reactivity.

Summa summarum, biochemists have become disillusioned with crystallography, and crystallographers have become impatient with biochemistry. It seems that a new technique is needed to provide structural information on short-lived (the

timescale of a biochemical reaction) intermediates along a reaction pathway. Such a technique needs the acquisition of diffraction data on a sub-second timescale. The successful application of such a technique is described in the work of Hajdu *et al.* reported in this issue. The method, which is 75 years old but has never previously been applied to protein crystallography, is called Laue diffraction (Friedrich, W., Knipping, P. & von Laue, M. *Sber. bayer. math. phys. Klasse (Kgl.) Akad. Wiss.* 303–322; Munich, 1912).

Conventional X-ray diffraction uses a single-wavelength X-ray beam. Diffraction from a crystal is observed when



The spots represent one plane of a lattice of reflections. A reflection is observed if it lies on the circle defined by the wavelength of the original beam. Thus, reflection A lies on the circle defined by $1/\lambda_{\max}$ and is observed if the incident beam has wavelength $\lambda_{\max}$. To observe all reflections, the lattice (the crystal) must be moved into all possible orientations such that the spots (reflections) cross the circle. With a polychromatic incident beam, there is a circle for every $\lambda_{\min} < \lambda < \lambda_{\max}$ and all reflections between the limits defined by $1/\lambda_{\min}$ and $1/\lambda_{\max}$ are observed with one orientation of the lattice.

Bragg's law is satisfied for any lattice point: $\lambda = 2d \sin \theta$, where θ is the angle of the incident beam to the diffracting plane, d is the interplanar distance, and λ is the wavelength of the incident X-ray beam. Traditionally, diffraction data are collected by varying θ and keeping λ fixed. The number of reflections satisfying the Bragg condition depends on the crystal orientation, and many positions are needed to obtain a complete data set. If a spectrum of wavelengths is used, then a larger number of reflections will satisfy the Bragg condition simultaneously (see figure) and, in favourable cases, a complete data set could be obtained with as little as one exposure. This is the principle of Laue diffraction. If the polychromatic X-ray beam is of very high intensity, such

as that produced by a synchrotron, the method has the potential for obtaining a data set on a very short timescale (Moffat, K., Szebenyi, D. & Bilderback, D. *Science* 223, 1423–1425; 1984). Complications arise because there are harmonic and spatial overlaps limiting the number of reflections that can be observed independently. Spatial overlaps can be resolved by choosing the orientation of the crystal and adjusting the crystal-to-film distance; harmonic overlaps comprise less than 17 per cent of any data set (Cruickshank, D.W.J., Helliwell, J.R. & Moffat, K. *Acta Cryst.*, in the press), so can be ignored.

The work described in the paper of Hajdu *et al.* in this issue clearly illustrates the feasibility of this method. The authors monitored the binding of the oligosaccharide maltoheptose to glycogen phosphorylase *b* by taking Laue data sets before, during and after the addition of the ligand. The synchrotron X-ray source at Daresbury in the United Kingdom provided a polychromatic radiation beam 0.2–2.1 Å in wavelength. Each data set required three independent X-ray photographs and took a total of 3 s to collect. Spatial and harmonic overlaps and selection of reliable measurement pairs reduced the unique data set to 25 per cent of the possible unique reflections, but even so, electron-density maps calculated from these data are clear and interpretable, comparable in quality to maps from monochromatic data. The half-saturation binding time for the system analysed by Hajdu *et al.* is about 8 min and so easily falls within the time constraints of the data collection. Maltoheptose can clearly be seen bound to the glycogen storage site of the enzyme.

Even with this breakthrough, a crystal-line enzyme system still has to meet certain criteria for the stages of a reaction to be followed using Laue diffraction. Transient intermediates, with half-lives of less than 250 ms, are still not observable, but any accumulating intermediate will be. It will be necessary to choose reactions which can be triggered in such a way that all molecules in a crystal are reacting at the same point along a reaction pathway within the timescale of successive exposures. There are several systems that meet these criteria, for instance, reactions triggered by photochemical means; by the introduction of a metal ion that diffuses rapidly into a crystal (Farber, G.K. *et al. Proc. natn. Acad. Sci. U.S.A.*, in the press); by a temperature jump; or by using mutants to slow down a reaction without altering its mechanism. Complete data sets with a single exposure are possible only if the enzyme crystallizes in a high-symmetry crystal form. Despite these limitations, the Laue method promises new insights into protein mobility and function. □

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Ozone depletion

Antarctic chemistry to blame

J.A. Pyle and J.C. Farman

OZONE depletion in the atmosphere is a cause for great concern, and the role of the Antarctic is of special interest. Last August to November, a collaboration of four groups, the National Ozone Expedition, made ground-based and balloon measurements at McMurdo, the first comprehensive survey of the distribution of trace constituents there. On page 126 of this issue, C.B. Farmer *et al.*¹ report infrared measurements that strengthen the conclusion, drawn from the work of Philip Solomon and co-workers (published recently in *Nature*^{2,3}), of Susan Solomon and co-workers⁴⁻⁷ and of Hoffman *et al.*^{8,9}, that chlorine is very important in the Antarctic atmosphere.

Many hypotheses have been advanced (see the recent News and Views articles by Margitan¹⁰ and Solomon¹¹), to explain the ozone hole over Antarctica. We shall restrict ourselves to the two extreme views: that depletion is caused by man-made chlorine pollution of the atmosphere; or that it is the result of natural atmospheric variations.

Most of the depletion apparently occurs⁸ in the first few weeks after the return of sunlight to southern high latitudes, during late August and September, large reductions occurring below 20-km altitude. A recent assessment¹² of the processes that control atmospheric ozone gave little indication that Antarctica is particularly sensitive to the growing burden of stratospheric chlorine. The main effects calculated to have occurred between 1960 and 1985 were confined to high altitudes, and were common to both polar regions. Reductions of column ozone by 50 per cent, as indicated by recent measurements¹³, were not anticipated to occur until well into the next century. In the models used in this assessment, the photochemical lifetime of ozone in the lower stratosphere was longer than six months. There is strong evidence, however, that the distribution of constituents over Antarctica in spring is radically different from that predicted by the models.

Chlorine abundance

Millimetre-wave measurements² of ClO suggest a markedly bimodal profile, with large concentrations below 20 km. OClO has been detected in concentrations 50 times larger than expected, showing clearly that the chemical scheme used hitherto is incomplete. The infrared measurements reported in this issue¹ indicate unusual partitioning of chlorine between HCl and ClONO₂. The column abundance of HF is three times larger than

that found at mid-latitudes¹². In the upper stratosphere, where chlorine is expected to be present mainly as HCl, the ratio HCl/HF is about 4 (ref. 12), having declined from about 10 since 1978. The ratio of (ClONO₂ + HCl)/HF at McMurdo on 12 October, was also about 4, so that the sum accounts for most of the chlorine at that time. In September the sum was much lower, suggesting⁸ that a large fraction of the chlorine was either in condensed phases, or in some other chemical form. The missing chlorine cannot be accounted for by ClO alone, as it was not detected spectroscopically¹.

Visible² and infrared¹ measurements show only small concentrations of NO₂, especially in mid-September. Changes in NO are well correlated with, but smaller than, NO₂ changes¹, a result consistent with the bulk of the NO being above the ozone layer. Nitric acid was twice as abundant in the column than at mid-latitudes. Large fluctuations of HNO₃, closely correlated with temperature, may indicate the presence of a condensed phase. Measurements of stratospheric N₂O taken with the Stony Brook millimetre-wave spectrometer are reported¹⁰ to show unusually low concentrations within the vortex, but a definitive account of these measurements has yet to appear.

Some have suggested that the ozone depletion could be a demonstration of the natural variability of atmospheric circulation, with the corollary that any year might bring about a recovery. Seasonal upwelling of air naturally poor in ozone would, indeed, account for the observed behaviour of column ozone. But this would be incompatible with the ozone profiles⁸, aerosol particle counts⁹, the high column abundance¹ of HF and the reportedly¹⁰ low values of stratospheric N₂O. Air rich in HF and poor in N₂O can only have come from a greater altitude. The mass transport occurs, we suggest, during the formation and intensification of the stratospheric vortex driven by the rapid cooling early in the polar night. Also, once formed, the core of the vortex is practically an isolated air mass, and persists as such until late spring¹⁴.

On this evidence, we believe that the ozone depletion is caused by the growing burden of halogens in the stratosphere. Depletion of radiatively active ozone should also give rise to dynamic effects, and these can now be discerned. To quantify them requires an understanding of the mechanical forcing of the vortex, which varies greatly from year to year¹⁵, and is largely responsible for the variable

timing of the onset of final warmings.

There has been much speculation about the polar stratospheric clouds detected¹⁶ by satellite. What is their composition? Are they simply passive phases or active sites for heterogeneous reactions? The outstanding issue, however, is the short photochemical life of ozone in the lower stratosphere over Antarctica in spring. A necessary condition for this is the virtual absence of the nitrogen oxides, which reduce the efficiency of the chlorine and hydrogen catalytic cycles. They cause null cycles involving the reactions of NO with ClO and HO₂, and form ClONO₂ and HNO₃. Although the recent observations show that this condition is satisfied, it is apparently insufficient. It seems necessary to invoke cycles for ozone destruction that are not rate-limited by atomic-oxygen concentration. The column abundance of HOCl could well be enhanced by the same mechanism as HF. The strong negative temperature dependence of the reaction between ClO and HO₂ helps to explain the distribution of ozone within the 'hole'. Other examples are cycles initiated by the mutual reaction of ClO radicals, and by the reaction between BrO and ClO.

Reaction cycles

Several groups are studying the important reaction cycles of BrO and ClO in the laboratory. Direct measurements¹⁷ of the product distribution and rate constants for the BrO + ClO reaction show that only two product channels are significant: (1) Br + Cl + O₂ and (2) Br + OClO. The branching ratio is 45:55, and the rate is invariant with temperature. Channel (1) leads to a catalytic cycle for ozone destruction of particular significance, because it requires neither sunlight nor atomic oxygen. The measured rate constant is smaller, by 45 per cent, than the previously recommended value. Channel (2) leads to a null cycle. It is, however, the only gas-phase reaction yet demonstrated to produce OClO. Some 20 parts per trillion by

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volume of bromine are required to explain the quantities of OClO measured⁶ at McMurdo. Bromine in this quantity could account, through channel (1), for a substantial fraction of the ozone depletion. These inferences are intriguing, but should be viewed with caution.

Preliminary studies¹⁸ of the dimerization of ClO show that this, too, could be a significant feature of low-temperature chlorine chemistry. Photolysis of the dimer¹⁹ leads to a cycle for ozone destruction, and, possibly, to production of

OClO. More data are required before the detailed mechanisms of the ozone changes can be identified unambiguously and discussion of ozone depletion, in Antarctica and globally, still involves much uncertainty. Nevertheless, the recent observations have changed the nature of the discussion irreversibly. □

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Hydrothermal activity

Venting events in hot water

Joe Cann and Ros Strens

In August 1986, in the course of routine surveys of the crest of the Juan de Fuca Ridge in the north-east Pacific, oceanographers discovered a gigantic thermal anomaly in the deep-ocean water. When they returned two months later it had disappeared. The chemistry of the anomalous water showed clearly that it had formed by mixing of very hot, hydrothermal fluid with normal cold ocean water. The observation, reported by E.T. Baker, G.J. Massoth and R.A. Feely on page 149 of this issue, clearly indicates a new kind of hydrothermal activity, very different from the steady venting of black-smoker fluids into the oceans to which we have become accustomed over the past ten years, although that in itself is a remarkable enough phenomenon.

In the normal black-smoker systems, very hot (350 °C) water emerges from the sea floor along the axes of mid-ocean ridges in discrete vent fields. The water carries dissolved iron, copper and zinc sulphides that are precipitated, when the acidic hot-vent water mixes with neutral cold sea water, to make chimneys and mounds of sulphides. Fossil analogues of such deposits are well known, and study of the active systems has been fruitful in advancing understanding of ore-deposit genesis.

The hydrothermal fluids involved originate as normal sea water that descends in the cold limb of hydrothermal convection cells and penetrates into reaction zones 1–3 km below the sea floor, where it is heated by hot rock or molten magma and reacts chemically with its surroundings, before being discharged as hot black-smoker fluid through rather narrow upflow zones. Discharge rates are probably in the range 1–100 kg s⁻¹ with corresponding heat flux (above ambient sea water) of 1.5–150 MW. The discharge forms a turbulent plume into which cold sea water is rapidly entrained and which rises to about 100–300 m above the vent field before spreading sideways as a layer about 100 m thick, marked by a temperature anomaly,

by particles of oxidized sulphide, by high levels of dissolved manganese and by other features characteristic of the black-smoker fluids, such as an anomalously high ³He/⁴He ratio.

Just such a normal steady-state plume can be seen on Baker, Massoth and Feely's Fig. 1 on page 149, which shows a north-south cross-section through the ocean above the crest of the Juan de Fuca Ridge near its southern end. The plume rises to about 200 m above the bottom, showing



A black smoker vent along the mid-ocean ridge.

that normal hydrothermal activity is taking place there. However, above the normal plume is a very different feature, a body of water, centred 800 m above the bottom, 600–700 m thick and 16 km across, with an astonishing temperature anomaly of more than 0.2 °C over a large part of its volume.

This body, the megaplume, contains a heat excess of about 7×10^{16} J above ambient sea water, equivalent to about 0.07 km³ of black-smoker fluid. Water samples from within the megaplume contained large grains of anhydrite (CaSO₄, precipitated from cold sea water when heated by vent fluids), chalcopyrite (CuFeS₂) and amorphous SiO₂, which would have settled out of the megaplume

within a few days. Baker, Massoth and Feely thus argue that the megaplume must have been no more than a few days old when they sampled it, and must have been created over a few days at most. To deliver so much heat so quickly, the mass flux of hot water must have been hundreds to thousands times that of normal black smokers (if it was at the same temperature), and the heat flux at least tens of thousands of megawatts. The fact that the top of the megaplume rose a kilometre above the ocean floor supports the high heat flux proposed.

What causes such a catastrophic emission of hot water? Baker, Massoth and Feely relate it to an episodic spreading event simultaneously injecting magma into a hydrothermal system and fissuring the system to allow hydrothermal fluid to escape suddenly. Certainly the amount of heat involved is about right, but there are potential problems, both with generating chemically more or less mature hydrothermal fluids and with transporting heat into the fluid fast enough. Heat transfer would be most effective in the type of violent submarine eruption that produces thermal granulation of magma into a glassy sand (hyaloclastites), and such eruptions are known from the geological record. It is very unlikely, however, that chemical changes would happen fast enough during such an eruption, as young hyaloclastites show little sign of water-rock reaction.

It is interesting that the volume of black-smoker fluid calculated as contributing to the megaplume (0.07 km³) is approximately the volume of hot water contained in a single hydrothermal system (as estimated from the volume of the high-temperature reaction zones in Cyprus; see Richardson, C.J., Cann, J.R., Richards, H.G. & Cowan, J.G. *Earth planet. Sci. Lett.* **84**, 243–253; 1987). Could the megaplume be the result of a catastrophic surge in the flow of a normal system that led to its emptying itself of hot water? We have predicted that such an instability could result from the rapid non-linear changes in the physical properties of sea water that occur at about 400 °C at sub-seafloor pressures (Cann, J.R., Strens, M.R. & Rice, A. *Earth planet. Sci. Lett.* **76**, 123–134; 1985). Systems might surge as part of a cyclic pattern rather than in response to tectonic events.

Whatever the answer, the fact that such a short-lived event as the megaplume has been discovered at this early stage of research into hydrothermal plumes suggests that megaplume activity could be a common, intermittent feature of hydrothermal systems. This would have major implications for biological activity, chemical flux into the oceans and sulphide precipitation on the ocean floor. □

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Population genetics

Observing the founder effect in human evolution

Jared M. Diamond and Jerome I. Rotter

IN evolutionary biology the concept of the founder effect refers to "the establishment of a new population by a few original founders (in an extreme case, by a single fertilized female) which carry only a small fraction of the total genetic variation of the parental population"¹. The resulting new population thereby instantly becomes genetically different from the parental population. Although the founder effect is often thought to be important in speciation, it has rarely been observed directly in nature, for the obvious reason that pedigrees of all individuals in an expanding population are generally unknown. In this respect, no other species can match the advantages of *Homo sapiens*: each individual of our species is named, our purported genetic relationships are known and our pedigrees can often be traced for many generations. Recent studies of human genetic disorders document the evolutionary significance of the founder effect in a way that would be impossible for other species²⁻¹⁰.

Consider the Afrikaner population of South Africa³, which was founded by a shipload of immigrants in 1652 and subsequently augmented by more immigrants. As documented by church registers, the early immigrants produced large families (often 10 or more children), underwent a population explosion and contributed disproportionately to the modern Afrikaner population, which now numbers about 2,500,000. Some original settlers left tens of thousands of descendants alive today, and nearly 1,000,000 living Afrikaners have the names of 20 original settlers.

Among the founding immigrants in 1652 were one carrier of the gene for Huntington's chorea and two carriers of lipid proteinosis, while carriers of porphyria variegata and familial colonic polyposis arrived a few decades later^{3,4}. In porphyria variegata, an autosomal dominant defect in the enzyme protoporphyrinogen oxidase, carriers develop a severe and sometimes lethal reaction to barbiturate anaesthetics, but the condition was relatively benign until the advent of modern medicine. All traceable Afrikaner carriers are descendants of one couple, Gerrit Jansz and Ariaantje Jacobs, who emigrated from Holland in 1685 and 1688, respectively⁴. Now, about 30,000 of their South African descendants carry the gene that they brought — a far higher incidence than in Holland — due to this sampling accident. Similarly, all South African cases of lipid proteinosis,

an autosomal recessive disorder that is more frequent there than anywhere else in the world, are traceable to a brother and sister who arrived in 1652³. One of the brother's five children and five of the sister's thirteen children carried the gene, as did eight of the seventeen offspring of the sister's eldest son, thereby propelling the gene towards its modern frequency.

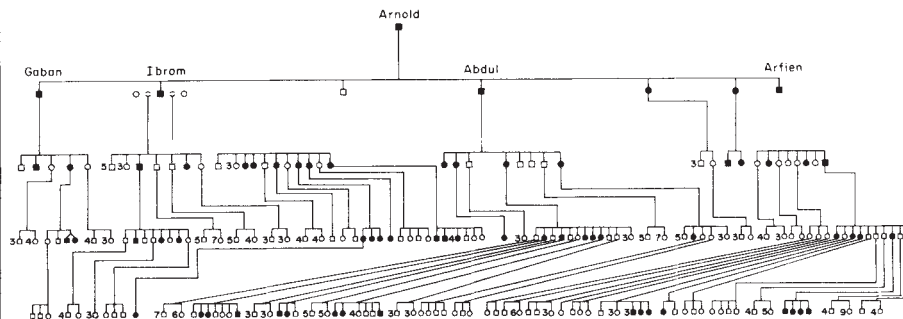
Although the founder effect is particularly easy to document among Afrikaners because of their church registers, there are many other examples in South Africa and in other parts of the world⁵⁻¹². In South Africa's Cape Coloured population, an autosomal dominant bone disease (osteodental dysplasia), causing complete loss of teeth by age 20, stems from a polygamous Chinese immigrant called Arnold who, aided by 7 wives, transmitted the syndrome to at least 70 of his 356 traceable descendants (ref. 5; see figure). As for Huntington's chorea, a lethal autosomal dominant disease, most Afrikaner cases derive from a Dutch man who arrived in 1652 and his German wife who arrived in 1668³; 432 cases in Australia are descended from the British immigrant Miss Cundick and her 13 children¹⁰; and all known cases on the island of Mauritius are descendants of a French nobleman's grandson, Pierre Dagnet d'Assigne de Bourbon⁹. All 82 cases of autosomal recessive six-fingered dwarfism in the Pennsylvania Amish community stem from Samuel King and his wife¹¹. Every human population has its own 'private' genetic disorders confined to or commonest in that population, and for which the founder effect must be considered as one possible explanation^{8,11,12}.

Naturally, deleterious genes eventually tend to be eliminated by natural selection, and the founder effect alone cannot explain their long persistence in a human

population. It is therefore interesting to examine what sort of geographically localized genetic disorders are still traceable to founder effects among Finns and Afrikaners after several centuries of natural selection. Some of the disorders are too benign to interfere seriously with reproduction. Those that kill their carriers are either autosomal recessive diseases (so that homozygotes die but the far more numerous heterozygotes remain unaffected) or autosomal dominants that affect adults only after they have already reproduced. For instance, the sixteenth-century population explosion in central Finland bequeathed to modern Finns a characteristic legacy of 19 genetic disorders¹³. Four are autosomal dominants or sex-linked recessives that are thus expressed in all or many carriers but mainly lead just to visual impairment in adulthood and thus do not prevent reproduction. Although the other 15 Finnish disorders do include lethal diseases of childhood, they are autosomal recessive conditions, so that heterozygotes are protected from effects of natural selection. Similarly, among the common genetic disorders of Afrikaners, those that are lethal before adulthood or interfere with reproduction are autosomal recessives (sclerosteosis, spondylo-epimetaphyseal dysplasia) whereas the autosomal dominants are either benign (porphyria variegata before the advent of barbiturates) or lethal only in post-reproductive adults (Huntington's disease, familial colonic polyposis)¹.

The founder effect can thus be shown to endow populations temporarily for a few centuries with high frequencies of benign traits or of deleterious genes that are relatively protected from the effects of natural selection. What do these esoteric diseases tell us about the evolution of our persistent 'racial' characteristics that are selectively neutral or advantageous, such as skin colour, blood groups and disease resistance?

Throughout most of human evolution our population structure made us prime material to be moulded by founder effects². Breeding populations of hunter-gatherers were small, of the order of 500 or less, and polygyny allowed a few



The founder effect in operation: how polygamy and fertility enabled one carrier of the gene for osteodental dysplasia to pass the gene to at least 70 descendants in the next four generations. Squares, males; circles, females; filled and open symbols, affected and unaffected individuals, respectively. (From ref. 5.)

successful males to skew the distribution of the next generation's gene pool. Tiny founder groups underwent massive population explosions in several large and formerly uninhabited areas of the globe, such as when the ancestors of American Indians, native (aboriginal) Australians and Polynesians reached their eventual homelands. Other founder groups underwent massive expansions at the expense of earlier inhabitants, such as when Indo-Europeans spread from the Ukraine over much of Eurasia; Austronesians spread from Taiwan over the Indo-Malayan archipelago; or Bantu speakers overran much of sub-Saharan Africa. These populations had to adapt to their new environments, but the genes that were initially available to them for adapting depended on the founder effect. This could help to explain why malaria resistance depends in different locations on many alternative genes, including mutant haemoglobins, thalassaemias and G6PD variants.

As an extreme case of the founder effect, the initial peopling of Australia from Indonesia 50,000 years ago probably

resulted from the accidental arrival of a raft bearing a few people — perhaps no more than a pregnant woman expecting a son¹⁴. Which of the distinctive features of modern native Australians go back to the genes of that woman? □

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Alzheimer's disease

Tangled genes and proteins

Brian H. Anderton

DEVELOPMENTS in studying the molecular biology of Alzheimer's disease are coming thick and fast. It is only a few months since it was announced that the gene responsible for the rare autosomally dominant familial form of Alzheimer's disease (FAD) is located on chromosome 21. At the same time, the gene encoding the precursor amyloidogenic protein was also found in close proximity to the gene encoding FAD, the two perhaps being one and the same. This result seemed to offer a neat explanation, by a gene-dosage mechanism, for the development of Alzheimer's pathology in people more than 40 years old with Down's syndrome (see my recent News and Views article¹). Results reported at a recent meeting², and in two papers by J.A. Hardy and colleagues³ and by J.F. Gusella and colleagues⁴ on pages 153 and 156 of this issue, respectively, show that the situation is not so simple.

During the past decade, research has concentrated on three pathological changes in the brains of patients with Alzheimer's disease: intracellular neurofibrillary tangles, made of paired helical filaments; extracellular amyloid deposits at the centre of senile plaques, the areas of neuronal degeneration; and deficits of neurotransmitters, their synthetic

enzymes and their receptors. Most cases of Alzheimer's disease are believed to be sporadic, although it is increasingly recognized that a familial tendency may be more common than was previously thought. In the few families in which FAD exists, however, the disease is usually more aggressive and has an earlier onset than the more common 'sporadic' or looser familiarly associated disease. Linkage analysis in four such FAD families where a large pedigree of inheritance is known is a new line of attack undertaken by Gusella's group in Boston, whose results were reported at the meeting by P. H. St George-Hyslop (Harvard Medical School).

The gene encoding FAD has now been more precisely mapped on chromosome 21 and lies close to the two markers *D21S16* and *D21S1/D21S11*. This region is outside the obligate Down's syndrome region, which is known because about 3 per cent of Down's cases do not have complete trisomy (three copies) of chromosome 21 but only of the part of the chromosome (the obligate region) at the distal end of the long arm. The gene encoding FAD is more proximal than this obligate segment. What is not known is if all Down's cases that have incomplete trisomy are demented and develop an Alzheimer's pathology, or if it is only those that have three copies of the FAD and/or amyloid-precursor gene.

Great excitement followed the finding

reported a few months ago that the FAD and amyloid-precursor (AP) genes are on the same chromosome and not too far apart (see ref. 1). However, St George-Hyslop, J. Hardy (St Mary's Hospital Medical School, London) and C. Van Broeckhoven (University of Antwerp) all reported studies of families with Alzheimer's disease in which the disease failed to co-segregate exclusively with one particular allele of the AP gene. They concluded that it is very unlikely that the FAD gene is identical with the AP gene^{2,3}.

St George-Hyslop also reported a careful re-examination of the recent claim⁴ that 'sporadic' cases of Alzheimer's disease result from a duplication of the distal portion of chromosome 21 that includes the AP gene. Both he and Hardy were unable to confirm this observation, and agreed that the AP gene is not responsible for Alzheimer's disease either in mutant form (FAD) or by the presence of an extra copy (sporadic Alzheimer's disease and Down's syndrome)^{2,3}.

These results imply that amyloid deposits are the consequence of other earlier events in the pathogenesis of the disease, the FAD gene perhaps encoding a protein that influences the processing of the amyloid precursor protein. The next step is therefore to study the biochemical changes that cause the *M*_r 4,000 amyloid A4 protein to be produced from its precursor. M. Salbaum (University of Heidelberg) reported that the precursor protein is a *M*_r 92,000 polypeptide of the particulate fraction from brain. The recombinant protein produced by *in vitro* transcription and translation of the complementary DNA is susceptible to proteolysis but can be protected from proteases by insertion into added microsomal membranes. The recombinant protein can also be glycosylated. All this suggests that it is a membrane glycoprotein as originally proposed by Salbaum and co-workers^{1,5}. Hardy found by Northern blot analysis that the messenger RNA encoding this protein is more abundant than actin messenger RNA in brain, which suggests that the *M*_r 4,000 A4 amyloid protein is produced by proteolysis of one of the most abundant brain membrane proteins whose identity, for the moment, remains obscure.

The chemistry of the other main protein deposit, the paired helical filaments of the neurofibrillary tangles, is not so well advanced. C. Wischik (Laboratory of Molecular Biology, Cambridge) described the identification of a polypeptide of *M*_r 9,000–12,000, probably a fragment of a larger protein, that is present in the core of the paired helical filament and accessible only after treatment of isolated filaments with proteases. This polypeptide does not seem to cross-react with any cytoskeletal protein, the other constituents of neurofibrillary tangles. M. Landon (Nottingham University) and I can both confirm

*Meeting sponsored by the Brain Research Association and the Alzheimer's Disease Society of Great Britain, Southampton, 22–24 July 1987.

that ubiquitin is a component of tangles^{6,7}, implying that tangles contain proteins that the affected neurons recognize as abnormal and try to remove via the ubiquitin pathway. Thus, tangles and amyloid deposits are probably derived by some aberrant post-translational modification of precursor proteins. J. Candy (Newcastle University) described results of investigations into how aluminium enters the brain, because the plaque core, in addition to deposits of A4 amyloid protein, also contains inorganic aluminosilicate. Aluminium seems to compete successfully with iron for binding to transferrin, and the transferrin receptor on IMR32 neuroblastoma cells will bind and take up aluminium-transferrin. Once on the wrong side of the blood-brain barrier, aluminium might produce aberrant protein metabolism with the production of A4 amyloid deposits and, possibly, paired helical filaments. This idea is speculative as aluminium does not normally cross the blood-brain barrier and it is first necessary to establish how it does so in people with Alzheimer's disease. One possibility could be by disturbance of the transferrin uptake system in brain capillary endothelial cells.

Finally, families and carers of sufferers should be considered in planning future research strategies. From a survey of carers, it is apparent that aggressive and antisocial behaviour of patients is more upsetting than their dementia, which can be more readily tolerated. It was suggested that the search for useful drugs should not concentrate exclusively on the cholinergic system, as it is also important to treat the different clinical symptoms, including the behavioural rather than cognitive changes. The histopathology is also quite variable and if this disease is fundamentally a genetic disorder, it is quite likely not to be a single type of genetic defect. St George-Hyslop pointed out that identifying the FAD gene and its function may not be the ultimate solution for treatment. Perhaps in addition to searching for pharmacological means of supplementing neurotransmitter deficits, we shall see the development of compounds to interfere with the enzymes associated with tangle and amyloid production, once these processes have been identified. And so, as J. Even-den (Merck, Sharp and Dohme, Harlow) commented, every pharmaceutical company could produce its own drug that might be useful for one aspect of the disease. □

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High-energy physics

An American Z^0 factory

A correspondent

Z^0 PARTICLES, one of the carriers of the nuclear weak force, were first discovered in the Nobel-prize-winning work at CERN in 1984. It should be possible, using them, to test many of the predictions of the so-called standard model of weak and electromagnetic interactions. At CERN, the Z^0 s are generated in high-energy collisions of protons and anti-protons, but only in a tiny fraction of all observable events. For more efficient production, collisions of electrons and positrons (anti-electrons) are needed. Hence the frantic race between CERN and Stanford Linear Accelerator Center (SLAC) to build their respective new electron-positron colliders, in which Stanford, perhaps by cutting corners, appears to have the edge.

At CERN, the large electron-positron (LEP) collider under construction is a conventional storage ring, except it is 20 miles in circumference. Initially the storage ring will have sufficient energy to produce Z^0 ; subsequently its energy will be substantially increased, to produce pairs of W^+ and W^- bosons. Construction has been delayed and commissioning is expected to start early in 1989, followed six months later by the first experiments.

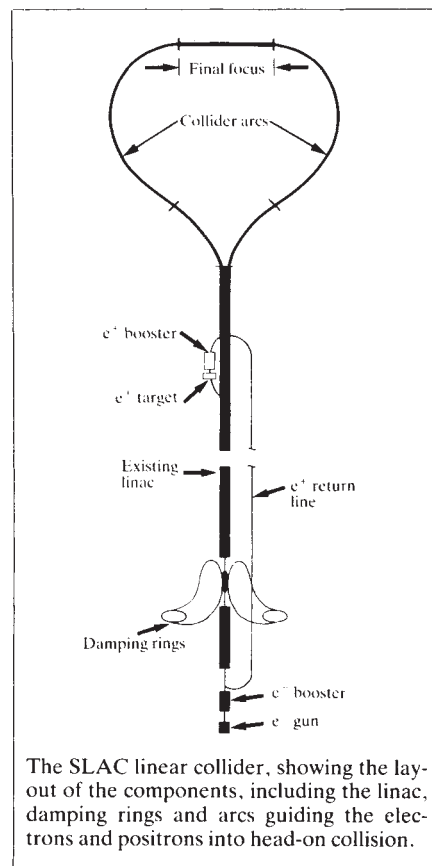
Engineers at CERN expect LEP to provide each of its four detectors with approximately 15,000 Z^0 s per day. Such predictions, however, are speculative and past experience has shown that the difficulties of operation of a storage ring increase with size and energy.

By contrast, the Stanford Linear Collider (SLC), at SLAC, is an innovative and bold (some call it foolhardy) short cut to Z^0 production based on the existing linear accelerator (linac). It has been a substantial drain on manpower and technical resources but is relatively cheap, costing only \$120 million; LEP will cost at least \$750 million. The projected rate of Z^0 production is somewhat lower for SLC than for LEP. On the other hand, unlike LEP, SLC will be able to provide polarized beams, making many unusual experiments possible.

The figure shows the layout of the Stanford collider. The existing SLAC accelerator provides the required energetic positrons and electrons. First, low-energy beams of electrons and positrons are injected into damping rings. These damping rings compress the electrons and positrons into intense bunches, that are injected into the linac and accelerated to an energy of 47 GeV. At the end of the linac, guide fields bend the beams round opposing arcs, two half circles added to the linac. Focusing magnets, either side of the interaction zone, compress the bunches yet further to just 1.5 μ m in diameter. After traversing the collision point the beam quality is poor and the bunches are steered out to a beam-dump.

With all the hardware in place, the commissioning of SLC has been proceeding for several months now, 2 years ahead of LEP. Z^0 production requires a doubling of the beam energy of the existing accelerator. This is achieved by compressing the radio-frequency pulses from the klystron accelerators by a factor of two, which leaves the pulse duration still much greater than the residence time of the electron or positron pulse. Thus the peak intensity of klystron pulse is doubled, increasing accordingly the acceleration of the beam.

But although SLC is operating reliably at the required energy, the electron bunch intensity is low by a factor of two and the positron intensity is low by a factor of ten. Furthermore, preliminary indications are that the required alignment of the hexapole and quadrupole magnets in the beam-transport system will be substantially more difficult to achieve than anticipated. Surprisingly, the operation one might



The SLAC linear collider, showing the layout of the components, including the linac, damping rings and arcs guiding the electrons and positrons into head-on collision.

expect to be hardest, the aiming of the beams at each other with micrometre precision, should be easy. Bunches passing close to each other are deflected by their mutual electrical attraction, causing them to emit a strong burst of photons (bremsstrahlung). The closer together the beams are, the stronger is the bremsstrahlung pulse, making the optimum alignment easy to identify.

The problems encountered at present mean that the rate of Z^0 production will not exceed several per day (one thousandth of the expected rate). Should the difficulties persist, full operation of SLC

could be substantially delayed, but as soon as SLC is producing several Z^0 's per day, experiments using the Mark II detector can start.

But even if SLC is the first Z^0 factory to operate it could be that if LEP runs to specification, its superior luminosity will allow physicists at CERN to mop up all the exciting results. Given the expense of, and technical investment in, the SLC and LEP projects, the outcome of the race could be crucial to the futures of both laboratories. The race is tinged both with the excitement of the discoveries to come and with the fear of coming second. □

Geology

Origin and growth of continents

Simon L. Harley

It is widely accepted that the recent growth of continental crust has occurred largely through the accretion of mantle-derived magmas, mainly andesitic, at island arcs or Andean convergent plate margins. At a recent conference*, the overall importance and characteristics of such subduction-related mechanisms in the past growth of continents were seriously questioned, and the significance of other processes, including hotspot or plume activity and magmatic underplating, were explored.

Past episodic and rapid-growth phases involving the accretion of dominantly felsic (feldspar and quartz) to intermediate crust generally imply accretion rates up to ten times those recently proposed for island arc zones¹. This has become recognized from studies of Archaean and Proterozoic tectonic belts, such as in Greenland from 3.9 to 3.6 gigayears (Gyr) ago, (P. Kinny, Australian National University (ANU) at Canberra), Western Australia, 4.2–3.9 Gyr (W. Compston, ANU), Swaziland from 3.6 to 3.45 Gyr (A. Kroner, Mainz University) and 2.9–2.7 Gyr (M. Whitehouse, Oxford University), and Churchill Province, Canada, 1.9–1.7 Gyr (ref. 2).

Underestimates

Although the earlier estimates of 1–2 $\text{km}^3 \text{yr}^{-1}$ probably underestimate recent accretion in the Andes by an order of magnitude (C. Wood, Johnson Space Center; P. Francis, Lunar and Planetary Institute), it is likely that much of this newly added material is basaltic in composition (R. Ellam, Oxford University; C. Hawkesworth, Open University). The andesites are considered to be products of intracrustal fractional crystallization of basalts, rather than primary mantle melts, and a voluminous, mafic (rich in mag-

nesium and iron) cumulate lower crust or keel is required.

The crustal composition generated in this subduction-related process shows a low Rb/Sr ratio. This contrasts with the high Rb/Sr ratio of the bulk crust and crustal additions in the major growth episodes. The felsic crusts are thought to have been formed by partial melting of basaltic precursors at mantle depths (W. Collins, MacQuarie University) and further remelting of the product tonalites.

Greater production of heat during the Archaean and early Proterozoic may have been accommodated by increased rates of plate formation and destruction through faster seafloor spreading or longer ridges (D. Abbott, Lamont-Doherty Geological Observatory; D. Howell, US Geological Survey) or by intense hotspot activity. The latter could have provided an additional mechanism for the growth of new continental crust. Rapid growth episodes could be produced by second- and third-stage melting in and above ascending plumes generated from instabilities at the core-mantle thermal boundary layer (G. Schubert, University of California, Los Angeles). The action of ancient hotspots might well be recorded in chemically distinct, 100–200-km-diameter, volcanic depletion cells (S. Haggerty, University of Massachusetts). The thickness of these cells would be about that of the lithosphere, and they could carry the eclogites that bear Archaean diamonds found in more recent kimberlites. The present lithosphere may in part consist of coalesced old depletion cells of laminated eclogite and depleted peridotitic mantle, along with later components.

Increasingly, it is being suggested that the main mechanism of crustal growth in modern-day Andean settings, Phanerozoic mountain belts, and Proterozoic rift-related terranes is the underaccretion of dominantly basic magmas. Studies of

lower-crustal xenolith suites in north-eastern Queensland (R. Rudnick, ANU) show that mafic granulites, emplaced at depth as basaltic magmas at 300 Myr and subsequently cooled without major uplift, comprise a large portion of the lower crust in that region.

The Keweenawan rift of the mid-United States is interpreted as a site of major crustal growth involving underplating of mafic magmas in the lower crust (P. Weiblen and J. Miller, Minnesota Geological Survey). It is apparent that the crust is added to from below in various tectonic settings; if these processes have always been dominant then the crust, and in particular the lower crust, should be rather mafic or cumulate in composition. An important question still to be answered is whether this is generally true or whether such mafic lower crust, especially after remelting and metamorphism, is recycled in some way into the mantle.

Recycling

The possibility of the continental crust being recycled is important because models for the growth of the crust can be well constrained only if recycling is negligible. Geochemical and isotope studies of incompatible elements, in particular lead and neodymium (S. Galer, Scripps Institute; S. Goldstein, Max Planck Institute, Mainz; R.K. O'Nions, University of Cambridge), convincingly demonstrate that pelagic sediment or material with typical, fractionated upper-crustal geochemistry is not effectively recycled into the convecting mantle. In the absence of recycling, the average neodymium age of the sedimentary record (Goldstein; P. Taylor, Oxford University) at 2.0–1.75 Gyr probably represents the average age of the continental crust and implies that about 50 per cent of the crust was present by that time and 90 per cent by the Phanerozoic.

This sedimentary bulk-age agrees with the geological and direct isotope data on the major growth episodes or pulses, provided that the early- and mid-Proterozoic episodes are at least as important as the late-Archaean (after 3.1 Gyr) accretion events. Both records indicate that crustal-growth models like those in refs 1 and 3 that include rapid early growth of 70–100 per cent of the crust before 4.0 Gyr cannot be correct, and that models which predict only 10–20 per cent growth since the Archaean¹ seriously overestimate the proportion of crust produced during 3.1–2.5 Gyr. The consensus is that growth, although pulsatory, is continuous, at least until the late Proterozoic.

Recycling of the lower crust and sub-jacent lithospheric mantle is, however, not well constrained by isotope and geochemical data. It is suggested from geophysical modelling (Schubert) and recognition of near-isobaric cooling paths in lower-crustal granulites (my own work)

* *The Growth of the Continental Crust*, 13–16 July 1987, Oxford University.

that detachment or delamination of the sub-continental lithosphere has been an important process throughout Earth history; such lithospheres may act as separate long-term source areas or be partly recycled into the convecting mantle. Detachment of lower-crustal to sub-crustal eclogitic residues (Collins) or contaminated mafic-ultramafic cumulates in the lower parts of magmatically thickened crust (N. Arndt and Goldstein, Max Planck Institute, Mainz) have also been proposed as mechanisms for the production of stabilized and broadly tonalitic crust or for buffering the crust-mantle system.

It is important that the significant geochemical and isotope characteristics of detachable crust and lithosphere are identified and their potential for recycling and mixing or retention as separate reservoirs

clarified. Clearly, if lower crust (such as the reflective lower crust of western Europe) and lithosphere can detach then our estimates of past growth based on upper-crustal isotope signatures and of average crustal composition will be too low. Models yielding 80 per cent of crustal growth by the end of the Archaean would again be viable if much of the recycled crust was relatively unfractionated and fed into the mantle by processes other than subduction. □

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Cell biology

Ubiquitous cycles of repair

Steve Sedgwick and Lee Johnston

How can one gene in yeast, *RAD6*, affect meiosis, resistance to DNA damage, induced mutability and the cell cycle? Even the cloning of *RAD6* two years ago¹ did not reveal how it plays such a central role in DNA metabolism. However, Jentsch *et al.*, on page 131 of this issue², report their quite unexpected finding that *RAD6* protein attaches ubiquitin to the histone H2B. This result means that a change in chromatin configuration can lead to complex changes in cell properties. Because ubiquitin is found in all eukaryotic organisms so far examined, the work of Jentsch *et al.* has important implications for many aspects of cell biology.

Ubiquitin is highly conserved, with only 3 of its 76 amino acids differing between yeast and mammals. It can exist free or covalently bound to various proteins, where one of its functions is to promote selective proteolysis of the protein concerned. The ligation of ubiquitin to target proteins occurs in a series of steps: first, ubiquitin joins to an activating enzyme, E1; second, activated ubiquitin is transferred to several conjugating enzymes, E2s; third, E2s donate ubiquitin to specific proteins, which sometimes requires a third enzyme, E3; and finally, isopeptidases remove ubiquitin from some protein-ubiquitin conjugates.

Jentsch *et al.* discovered² that the yeast *RAD6* gene encodes an E2-type conjugating protein that ubiquitinates H2B *in vitro*. They began by sequencing a fragment of a yeast E2 protein, and were surprised when the 14 amino acids of this fragment turned out to match the previously published amino-acid sequence of the yeast *RAD6* protein¹. The authors

then expressed the entire *RAD6* gene in *Escherichia coli* and showed that cell extracts acquire H2B-conjugating activity.

Jentsch *et al.* speculate that ubiquitination of histones, and perhaps other basic chromosomal proteins, by the *RAD6* gene product *in vivo* induces what they call 'chromatin remodelling', a process that would allow access of repair enzymes to DNA lesions. Such targeting of repair to damaged regions could occur if chromatin which is already ubiquitinated becomes more susceptible to remodelling around DNA damage, or conversely, if DNA lesions trigger localized histone ubiquitination. There are two possibilities for the remodelling step itself. The first invokes the known role of ubiquitin in tagging proteins destined for proteolytic degradation. The second is that the ubiquitinated histones remain in place but with modified properties.

Thus, chromatin remodelling is either the result of proteolytic removal of certain histones or the direct consequence of structural changes produced by ubiquitination. Changes in chromatin structure of this sort could also affect the accessibility of DNA to replication, recombination or even transcription. Indeed, there is a disproportionate amount of ubiquitinated chromatin in fruitfly and mammalian sequences which have the potential to be highly expressed³. On expression of such genes, chromatin dissociates, presumably to permit transcription. It is tempting to speculate that the ubiquitination that marks out a transcribable sequence is also involved in bringing about the preferential repair that has been detected in expressed mammalian genes⁴. Until the full range of

substrates for *RAD6* ubiquitination is known, there should be some caution in trying to explain all *RAD6* activity in terms of chromatin remodelling. Ironically, the highly acidic tail of *RAD6* protein had already led to the suggestion¹ that it remodels chromatin, although by the different mechanism of competitive binding with histones.

A role for *RAD6* in the cell cycle is suggested by the properties of mouse ts85 cells, which have a temperature-sensitive E1-conjugating enzyme and stop dividing at the G₂ phase of the cell cycle at restrictive temperatures⁵. The E2-conjugating activity provided by *RAD6* may also affect cell division because of the slightly protracted S and/or G₂ phases of *RAD6* mutants⁶. Expression of *RAD6* seems to be confined to the G₂ phase of the cell cycle⁷, suggesting that its usual role is in DNA synthesis or mitosis. It is also possible that other cell-cycle mutants, for instance some in the yeast *cdc* collection, are defective in ubiquitination. It might even turn out that there are oncogenes with defective ubiquitin metabolism.

As far as DNA-repair studies are concerned, ubiquitination is a totally new and exciting factor. Given the apparent conservation of ubiquitin metabolism, it is likely that *RAD6*-like activities exist in mammalian cells. Thus, defects in ubiquitination could account for the pleiotropic phenotypes of some human syndromes. It is a fairly safe bet that many DNA metabolism mutants will soon be taken out, dusted off and assayed for defects in ubiquitin metabolism.

The results described here emphasize the importance of post-translational modification of proteins in the regulation of cellular processes. Jentsch *et al.*² had the good fortune to have characterized a mutant in ubiquitin metabolism whose phenotype is already richly detailed. They will now need to find out whether all the functions of *RAD6* are exerted solely through ubiquitination of H2B, or if other undescribed proteins are involved. Whatever the outcome, the unravelling of the complex phenotype of *rad6* mutants in terms of defective ubiquitination should reveal basic rules about how this type of post-translational modification controls the behaviour of cells. □

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Archaeology

Excavation of a palaeolithic plank from Japan

Paul G. Bahn

THE palaeolithic occupation of Japan has become well documented in recent years through a series of excavations that have uncovered various stone-tool industries^{1,2}, and finds such as the small, flat engraved pebbles, some depicting apparent 'breasts' and 'skirts', from layer IX (Initial Jomon) in the cave of Kamikuroiwa, dated to 12,165 before present (BP)³ and, of course, well-authenticated finds of pottery dating back at least 12,000 years⁴.

One of the enigmas of the period was the human innominate bone, the large bone comprising the lateral half of the pelvis, found in 1931 by Nobuo Naora in eroded cliff material at Nishiyagi, near Akashi city, central Japan. Because of its degree of fossilization, the bone was originally attributed to the Middle Pleistocene, and constituted the first discovery of a pleistocene occupation of Japan. Many scholars, however, believed it to be a modern intrusion into the Nishiyagi Formation. Unfortunately the bone was destroyed by fire in the Second World War, but recent metrical studies of replicas have resulted in conflicting theories, some attributing the bone to *Homo erectus*, others to modern *H. sapiens*. In an attempt to obtain a precise age for the bone, Hideji Harunari and Toyohiro Nishimoto of the National Museum of Japanese History led an excavation in March 1985 (ref. 5).

According to geologists, the Nishiyagi Formation comprises a valley, eroded out during the Riss glaciation and then buried during the Riss/Würm interglacial. The bone is thought to have come from a layer of alluvial gravel and sand, 3.13 m above sea level. The excavation was done a few metres away; the upper 8 m of the 12-m cliff at this point were removed mechanically and the next 2.4 m excavated by hand. Unfortunately, no more bones were found, and no stone tools; but to their great surprise the excavators uncovered a wooden board in this layer, preserved by waterlogging. The find was first announced in 1986 (ref. 6), and I was privileged to be able to examine it in the museum laboratories, through the kindness of Professor Harunari.

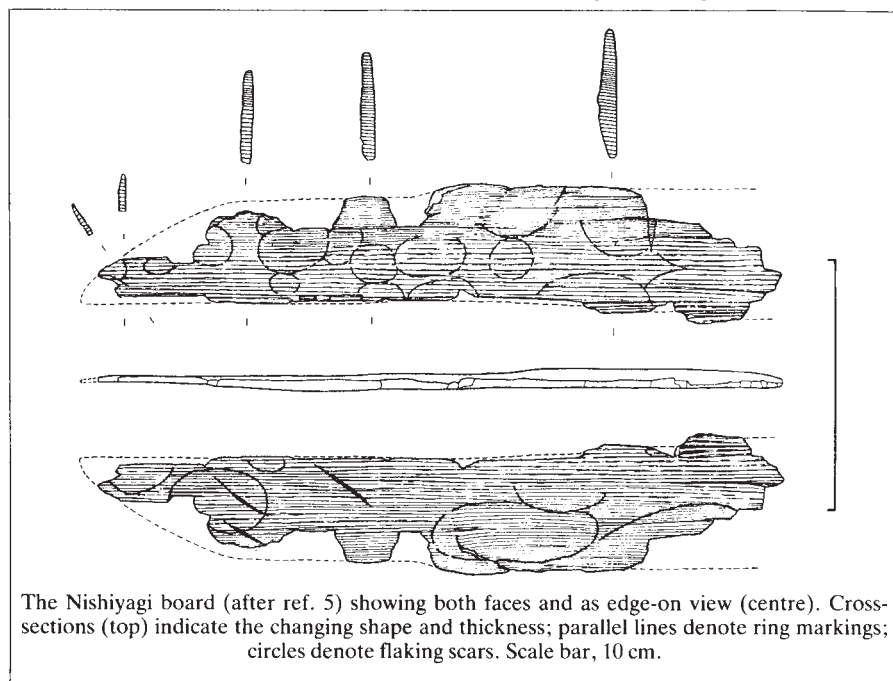
The board is thin and narrow, and has clearly been shaped, flaked and perhaps polished. The surviving fragment (26.9 cm long, up to 5 cm wide and 3–7 mm thick; see figure) is certainly artificial: it was cut across the tree rings, probably by splitting a trunk vertically with wedges, and was

then whittled into shape. Both sides have clear oval indentations, like fish-scales, which indicate further shaping and working. One end, which comes to a sort of point, is thinner than the rest and this has been suggested to denote a function as a spear point or short sword, although this seems unlikely in view of the object's thinness.

The wood has been identified as a species of mulberry (*Cudrania tricuspidata*) which is extinct in Japan (except for specimens imported during the past century) but which still grows in China and Korea, and indicates a warm climate. The

How old is the Japanese board? The possibility of modern intrusion was examined but firmly excluded. Dating of the layer is imprecise, but a combination of different methods points to somewhere between 50 and 70,000 years BP. The geomorphological and palaeomagnetic estimates of the Nishiyagi Terrace are 60–80,000 BP. Radiocarbon dating of wooden fragments from the layer, using accelerator mass spectrometry, gives a very rough date of about 54,000 BP. Finally, a flake of jasper which was found in the same layer to the west, and another found further along the cliff outcrop in 1958, closely resemble tools from the Babadan sites, Miyagi Prefecture, which are dated to 50 or 60,000 BP, as well as some middle Palaeolithic tools in China and Siberia.

Japan as yet has no defined middle Palaeolithic — merely an 'early' and a 'late'. The 'early' Palaeolithic, on present evidence, spans the period from about



The Nishiyagi board (after ref. 5) showing both faces and as edge-on view (centre). Cross-sections (top) indicate the changing shape and thickness; parallel lines denote ring markings; circles denote flaking scars. Scale bar, 10 cm.

board was cut from a tree at least 40 years old, which may therefore have been more than 8 m in height.

The working of the board implies that the makers had considerable knowledge of this raw material, which is hardly surprising as ethnographic studies suggest that many palaeolithic stone tools were used for woodworking; this hypothesis is confirmed by microwear analyses which prove that stone tools were used for sawing and cutting wood as far back as the Lower Palaeolithic⁷; and wooden artefacts, although rarely preserved, are known from various areas and palaeolithic cultures: for example, the Clacton and Lehringen spears and Königsau fragments in Europe⁸, or the Kalambo Falls material and decorated rods from Border Cave in Africa.

200,000 to about 30,000 BP, and therefore the wood must be assigned to the later part of that period. More precise dating results are expected in the near future, which may also help to elucidate the problems of whether 'Akashi man' was an archaic or a modern form of *Homo sapiens*. □

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Here be Komodo dragons

SIR—For as long as mankind has been discussing dragons, it has been difficult to distinguish fact from fiction; the recent article by Diamond¹ concerning the very real Komodo dragon is no exception.

The giant monitor lizard of Pleistocene Australia, *Megalania prisca*, although not well known, is not likely to have reached 2,000 kg in body weight. Neither of the references cited by Diamond support this figure. I am aware of only two quantitative estimates. Rich and Hall² (cited by Diamond) suggested that *Megalania* could have had eight times the mass of the Komodo dragon. The largest reported dragon³ was apparently 132 kg, therefore *Megalania* might have just exceeded 1,000 kg. Hecht⁴ estimated a lower figure of 600–620 kg. Both of these figures are within the normal range for large crocodiles of similar dimensions — for example, *Crocodylus porosus* and *C. niloticus*^{5,6}.

Megalania was not the only large carnivore in Australia during the Pleistocene. It has been suggested⁷ that the ziphodont crocodile *Quinkana fortirostrum*, which was possibly terrestrial, shared this niche with *Megalania* and no doubt *C. porosus*. The possible importance of large pythons such as the 5-m-long *Wonambi naracoortensis*⁸ should not be discounted.

As to the problem of what oras ate before the arrival of humans and their associated mammals, Diamond has confused the approximate dates for the introduction of domesticated species with the minimum estimates for the arrival of man. Humans were present in the region at least 35,000–40,000 years ago⁹ and in Australia are known to have shared the continent with the megafauna until about 6,000 years ago¹⁰. Because fossil dragons are recorded from the Pleistocene in both Timor and Java it is reasonable to assume that any of the large animals found in

these and adjacent islands may have been available as prey. These include¹¹; *Elphas* as well as *Stegodon*, a number of pigs, the pig deer (*Babyrousa babyrussa*), endemic 'wild cattle' (*Anoa depressicornis*), several large rats and a giant tortoise (*Geochelone atlas*).

If indeed Wallace had been able to visit the region at any time between perhaps 6,000 and 50,000 years ago, he would have found humans as well as dragons. In Australia the dragons probably shared the top carnivore niche with crocodiles of similar size (about 1,000 kg) and in Wallacia their diet was possibly more varied than just pygmy elephant.

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Disputed African origin of human populations

SIR—In a recent paper, Cann *et al.*¹ proposed a fascinating interpretation of the comparison between mitochondrial (mt)DNA polymorphisms in different geographic human populations. Their data certainly improve our knowledge of the molecular evolution of humans. However they do not provide a conclusive demonstration of the African origin of *Homo sapiens sapiens*.

The most important reason for this is that their parsimonious tree is not a phylogenetic tree: ambiguous character polarities do not yield safe trees². Cladistic approaches^{3,4} have shown that to be phylogenetic, a tree must be rooted after an estimate has been made of which are ancestral, and which derived, states. The widely used out-group criterion gives the polarity and is suitable for computing programs. In this system, a character state shared by both in-group and out-group is primitive. Unfortunately, for the populations of *Homo sapiens sapiens*, first, we do not have any out-group comparison; second, we do not know in a DNA sequence if a given base is primitive or derived, and third, the mtDNA polymorphism frequencies in populations are estimated from very small samples, for

example by using 18 black American and 2 African individuals to estimate the genetic diversity of the African population as a whole.

Even by using the 'midpoint rooting technique' the authors cannot convert their unrooted network of different mtDNA types into a phylogenetic tree of the human populations. From a methodological viewpoint, the midpoint method has certain flaws. For a given data matrix, the midpoint technique does not always give the same tree as the phylogenetic tree rooted either by an out-group or an ancestor. If a supplementary terminal taxon with only primitive characters (that is, identical to the ancestor) is added to the analysed taxa, it does not necessarily appear in an external or ancestral position in the tree rooted by the midpoint method.

No single hypothesis is certain as long as there is a possible alternative that cannot be proved to be false. Many other interpretations of the data of Cann *et al.* are possible as long as multiple hypotheses exist about relative rates of evolution along the branches of the tree, and as long as selective pressures, admixture, migration and bottleneck effects cannot be included in intraspecific models. To test such models, more than 9% of the mtDNA of 147 individuals must be tested, the mtDNA itself being 0.048% of the whole human genome. In this field, the best is yet to come.

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CANN *ET AL.* REPLY—Saitou and Omoto¹ argued that comparisons to mitochondrial (mt) DNA do not yield reliable trees relating populations to one another. We agree that population trees can be unreliable, especially when only a few individuals are sampled per population and when the extent of mtDNA divergence within populations nearly equals that between populations. For these reasons, we chose not to present a population tree in the article²

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Elephantos et Dracones

Caius Plinius Secundus: Editor Naturae.
have received a copy, sir, of...
your recent publication...
CONTAINING AN ACCOUNT OF THE...
RELATIONSHIP BETWEEN ELEPHANTS...
AND DRAGONS IN LANDS TO THE...
EAST OF INDIA. I thought I should
draw your attention to a passage
in my *Naturae Historiae*,...
libro VIII, that you may communicate
it to professor Diamond.
...sed maximo indubitanter cum his
perpetua discordia dracones canesque
michinus et ipsos de circumplexu pacis...
ambiant neque nudi praesentiant. com-
municare ex diplomata vicissim compens...
complexum elidit pondera.

AS TO DRAGONS IN AUSTRALIA, I KNOW NOT...
OF THAT PLACE, SIR, BUT IF THERE BE DRAGONS,
THEN SUPPLY TOO ELEPHANTS?

¹ with assistance from P.B. Mitchell, School of Earth
Sciences, Macquarie University, NSW 2109, Australia.
² Diamond, J.M. *Nature* **326**, 832 (1987).
³ Rich, T. & Hall, B. in *Vertebrate Zoogeography and Evolution*
in *Australasia* (eds Archer, M. & Clayton, G.) 391–394
(Hesperian, Sydney, 1984).

criticized by Saitou and Omoto. Rather, we published a tree relating mtDNA molecules to one another. Molecular trees can be reliable if many sites are compared, as was the case in our study of 370 sites in each mtDNA. This molecular tree allowed us to trace all known human mtDNAs back to one mother who probably lived in Africa about 200,000 years ago².

To cast doubt on this hypothesis, Saitou and Omoto¹ built a population tree using genetic distances taken from our paper. The significance of their tree, which does not support an African origin, must be questioned for two reasons. First, the sample sizes were too small for accurate estimation of genetic distances. Second, instead of using the actual data (in Fig. 3 of ref. 2) they used the genetic distances in Table 1, which had been rounded off to one significant figure. The use of the rounded off numbers heightened the uncertainty in their tree.

We have increased the number of New Guineans sampled from 26 in the original article² to 55 (ref. 3) and then to 119 (ref. 4). Genetic distances between New Guineans and other populations are now more accurate. The resulting population tree shows that the African population is most divergent from other populations, whereas the New Guinea population is related most closely to that of Asia, as Stoneking *et al.* have noted³. This population tree contradicts the main claim of Saitou and Omoto¹ and supports the proposed African origin for human mtDNAs².

As for their criticism of our mitochondrial time scale for human evolution, Saitou and Omoto¹ display no awareness of the primary paper on temporal calibration³, even though it was cited fully and explicitly². They also misinterpret our estimate of the mean rate of divergence of human mtDNA. The value we proposed, 2–4% per million years, refers to the comparison of two lineages, not as they supposed to evolution along one lineage. Therefore, their criticisms of the time scale and its implications lack substance.

Finally, we are puzzled by Saitou and Omoto's claim¹ that, according to Cann *et al.*², the African population diverged from other populations 200,000 years ago. We intentionally made no statement about times of divergence between populations because the concept of such a time is nearly meaningless for populations that still exchange genes. Perhaps our critics have not broken free from the constraints imposed by thinking in terms of gene frequencies, genetic distances, population trees, and time of divergence between populations. Studies of mtDNA have introduced a new way of looking at evolution below the species level^{5,6}.

Darlu and Tassy⁷, too, generate confusion when they criticize molecular trees as if they were population trees. Nevertheless, their main point is valid. Because

the molecular trees² for mtDNA maps and D-loop sequences were rooted by the mid-point method, it cannot be certain that the mitochondrial mother of us all was African. For this reason, we merely stated that "Africa is a likely source"². The case for an African origin, however, is not as weak as Darlu and Tassy⁷ claim. Their criticism neglects two justifications for the mid-point root, both of which foster the view that human mtDNA evolution is clock-like.

First, most (70%) of the surviving point-mutational differences found among human mtDNAs seem to be neutral⁸. Hence, their accumulation is expected to be mainly a function of time. Because, according to three separate studies^{2,9,10}, the African population is more variable than any other human population tested, it is likely to be the oldest. Second, the observed rates at which point mutations accumulate on surviving mtDNA lineages are alike in the three human populations subjected to rate tests³, namely aboriginal New Guineans, Australians and Americans. Furthermore these rates³ are not lower than in other mammals^{5,11} and birds¹². Such findings give empirical support for mid-point rooting.

Johnson *et al.*⁹ have made an *ad hoc* argument that the evolutionary rate may be higher on the primary branch leading exclusively to African mtDNAs, types 1–7 in the tree for mtDNA maps². If this were so, the midpoint method would falsely put the root on the African side of the true root. Although current data do not rule out an African acceleration, we should be aware that this argument has a corollary, which is that the average rate of human mtDNA evolution would be higher than we estimated^{2,3}. As a consequence, our previous estimate of 200,000 years ago for the time when the common mitochondrial mother lived^{2,3} would have to be revised toward an even more recent time.

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Receptor-gene sequence

SIR—In their paper giving the partial genomic sequence of V α 112–2 (part of the gene coding for the α -chain of a mouse T-cell receptor)¹, Hochgeschwender *et al.* located the 3'-end of an intron in an extremely unlikely place. Preceding the canonical AG at the splice site is a run of 15 nucleotides of which 8 consecutive ones are purine nucleotides and only 4 are pyrimidine nucleotides. It is well established² that purines are few and generally far between in this situation in other vertebrate introns. A little further upstream there is a sequence Py₂₈ GCACTGTAG which is much more likely to be the 3'-end of an intron. If this splice acceptor site were used, the reading frame of the presumed RNA would still be preserved and there would be an insertion of five amino acids in the protein coded for (Gly-Arg-Thr-His-Gly-) preceding the Asp residue as shown in Fig. 4 of Hochgeschwender *et al.* The Gly-Asp linkage that would occur in this putative protein is a likely linkage for cleavage by the signal peptidase to produce the mature protein.

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Correction needed

SIR—In my reply (*Nature* **328**, 675; 1987) to Collett and Loudon (*Nature* **326**, 671; 1987), lines 4–6 at the top of column three should have read: . . . that my prediction remains tenable but that the prediction of the Copenhagen interpretation *no longer* clashes with mine (as it does in the case of a "fixed source"). Unfortunately you omitted the words now put in italics.

If you allow me to add a comment on Collett and Loudon's reply that follows my letter, I would point out that in their original criticism they speak of a "fixed source", whereas in their new criticism they replace this by a "massive source". To my mind this means a change of the problem: they never explain why a (non-massive positronium) source cannot be "fixed".

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Scientific Correspondence

Scientific Correspondence is intended to provide a forum in which readers may raise points of a scientific character. They need not arise out of anything published in *Nature*. In any case, priority will be given to letters of less than 500 words and five references. □

Thoughts of tomorrow

Stuart Sutherland

Man-Made Minds: The Promise of Artificial Intelligence. By M. Mitchell Waldrop. Walker, New York: 1987. Pp.280. Hbk \$22.95; pbk \$14.95.

Thinking Machines: The Search for Artificial Intelligence. By Igor Aleksander and Piers Burnett. Knopf/Oxford University Press: 1987. Pp.208. Pbk \$17.95, £15.

AMONG other grandiose forecasts, made in the infancy of artificial intelligence (AI), was that we would have to make computers sentimental about the past so they would retain us as interesting pets. Since then, the subject has quite suddenly come of age. Ten years ago, there was only a handful of universities where the subject was taught; the first textbook of AI did not appear until 1975. Today there are dozens of texts and new departments incorporating the subject are opening every week. In their historical reviews of AI Waldrop, and Aleksander and Burnett, provide two main reasons for this sudden access of enthusiasm.

The first is the construction of AI programs that actually do something useful. The earliest example, DENDRAL, was developed in the late 1960s to help in the identification of chemical compounds from their profiles in a mass spectrometer. It departed from most previous work by incorporating knowledge of a particular domain, whereas many earlier programs had concentrated on abstract rules of inference.

The new breed of programs, which multiplied throughout the 1970s and 1980s, came to be known as 'expert systems' or 'knowledge-based systems'. The knowledge base incorporates the knowledge of human experts stored in a standard form (often that of the predicate calculus). When the program is presented with a problem, it applies to it the general rules in its knowledge base, many of which are usually probabilistic, and cranks out an answer. The reasoning involved is performed by a separate part of the program, the inference engine, which can draw deductions by, for example, using the rules of the predicate calculus. Most such systems can also request more information from the user if it is needed and can be interrogated to discover how a particular conclusion was reached. The latter facility is important because mistakes can occur for two overlapping reasons. First the program is limited to the information supplied by the programmer, and second the program has no common sense because the domain within which it operates is so restricted.

Perhaps the best known such program is MYCIN, which diagnoses and prescribes treatments for bacterial infections from analyses of bodily humours such as blood

or urine; although, according to one study, it performs better overall than almost every doctor, its lack of common sense can produce disastrous mistakes. The expert systems now in use include other medical systems, a geological system to advise on whether a given site may contain commercially exploitable deposits of ore and — perhaps the most successful of all — a system devised by a computer firm to advise on the most compatible configurations or optional components of its machines.

The second reason for the sudden expansion of AI in the West is not unconnected with the first. Impressed by the potential commercial use of expert systems, the Japanese Ministry of International Trade and Industry announced in 1981 the launching of a ten-year coordinated project costing a billion dollars to create the 'Fifth Generation' computer. The four previous generations had consisted of hardware advances, which were successively the vacuum tube, the transistor, integrated circuits on silicon chips, and very large scale integrated circuits on

a single chip, one of which could form a complete microprocessor. These devices brought vast increases in speed and storage space and an equally vast decrease in costs, all of which made possible the advances in AI. Although in their Fifth Generation machine the Japanese were intent on carrying these hardware developments further, they also put forward some staggeringly grandiose software objectives which included machine translation, the creation of knowledge bases on the scale of the *Encyclopaedia Britannica*, and the ability to read, converse in a natural language and understand pictures.

Although many of the more ambitious objectives were abandoned only a few years later, it was felt at the time that if the practically minded Japanese thought they could achieve such lofty targets, there must surely be more to AI than had previously met the eye. The British launched the Alvey program and the EEC reacted with ESPRIT: these programs were to coordinate and fund research at respective costs of one-third of a million and nearly a billion pounds. The United States also responded with increasing funding of AI under both defence and civil budgets. With such riches available it is small wonder that there has been a sudden stampede into the subject.

Two questions can be asked. First, is it a rush of Gadarene swine? So often in the past, the advocates of AI have made promises that were not fulfilled. As far as expert systems are concerned, the technology is well developed and they are



Soft shoe shuffle — a woodcut from Jakob Kobel's *Geometrie* of 1531, showing how the measure of one rod was determined by lining up 16 men, heel to toe, in soft leather shoes, as they emerged from church. The illustration is taken from *The Weights and Measures of England* by R. D. Connor, just published by HMSO, London, price £30.

likely to be increasingly useful. They are, however, of little intellectual interest. Indeed it is characteristic of the computer revolution that the most successful applications of computers — computer-aided design, airline reservations, word processors, bank transactions and so on — were devised without the need for ideas of much depth or generality. If you want to land an aircraft automatically you do not place in the cockpit a robot sensitive to light of between 380 and 740 nm with limbs to manipulate the controls: you simply fly the plane down a beam, a dull but effective method.

This raises the second question. Will the successful applications of AI divert its practitioners from more fundamental research such as obtaining a rigorous understanding of human vision, natural language and reasoning? The answer here must surely be negative. The commercial appeal of the discipline will continue to force expansion of AI departments. Although this had led and will for a time continue to lead to many of their staff being rather mediocre, within those departments the best brains are likely to continue to work on the most interesting and fundamental problems. Indeed in the long run — and it probably is a very long run — easy communication between man and machine can only be achieved once we reach a much better understanding of how the mind works.

Neither Waldrop nor Aleksander and Burnett raise the latter two questions, but each book is of value in its own way. *Man-Made Minds* gives quite a comprehensive history of AI and describes reasonably clearly a fair sample of work in the subject. The book is well documented, but has a marked American bias. Waldrop wrongly attributes the concept of an augmented transition network to an American rather than to Jimmy Thorne and his collaborators in Edinburgh, and he makes no mention of Max Clowes of Sussex University who wrote one of the first scene-analysis programs. Moreover, Christopher Longuet-Higgins may well feel indignant at being described as a neurophysiologist.

Waldrop is also a little too prone to accept programmers' claims at face value — as far as I know there is no evidence that LOGO or any other computerized educational system is more beneficial to students than normal methods of instruction. He is, however, particularly good on recent applied developments like the use

of 'Outlining' programs for planning: such programs enable the user to make notes of proposed activities and to shift around their order and interconnections. It is a compliment to Waldrop that although he claims to have used such a system in preparing his own book, the reader would not know it.

Thinking Machines contains a less detailed historical account, but it gives the reader a clearer and more detailed picture of the techniques of AI, including many procedures, such as breadth first search and the use of push down stacks, that are not mentioned by Waldrop. Two chapters are devoted to Aleksander's own work on pattern recognition: curiously enough, they are the only chapters in the book that are unclear.

Both books are useful. For a general account of AI including its history and politics read *Man-Made Minds* and for an

unusually clear elementary account of its techniques read *Thinking Machines*.

Both finish with discussions of the future, of which Waldrop's is the fuller. He considers such problems as the futility of human existence in a world run by robots, the ethics that should be implanted in them and the possibility — which he rejects at least for the near future — that they will throw people out of work. Aleksander and Burnett are more down to earth and cautious. They summarize their position neatly by taking up the issue of people as pets: "What we should be looking forward to is not the day machines start to treat us as pets, but a time when they will have become sufficiently intelligent for potential users to give them house room". □

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Pro-am araneology

John Dalingwater

The Spiders of Great Britain and Ireland, Vols 1-3. By Michael J. Roberts. *Harley Books, Martins, Great Horkesley, Colchester CO6 4AH, UK: 1985 and 1987. Vol. 1 pp.229, £45; Vol. 2 pp.204, £45; Vol. 3 pp.256, £55. £135 the set.*

PALPAL structure in male spiders and the appearance of the genital area in females are usually extremely complex and species-specific. So, crucial to any diagnostic account of spider species is the quality and accuracy of the illustrations of palps and epigynes: in Roberts's work these are of a standard rarely achieved in the past and unlikely to be matched in the future. The recently published Vol. 2 on the Linyphiidae, the last of the three volumes to appear, is particularly welcome. It will greatly ease identification within this family, which contains nearly half of the 623 British species.

Why, therefore, will these books not be at the elbow of every serious British araneologist as he identifies his spiders? There are two, interconnected reasons: size and price. These A4 volumes are difficult to use alongside a microscope and are so expensive that those fortunate enough to be able to afford them may not wish to risk their investment on the work-bench. I suspect that the books are so expensive because their large and lavish format was determined by the requirements of the least useful, though admittedly the most spectacular, volume — the third. This consists mainly of full-page colour plates of selected species, painted from freshly preserved specimens; they are splendidly detailed and accurate, a joy to behold, but of limited value for identification.



Eight-legged art — male *Oxyptila praticola*, from the colour original in Vol. 3 of Roberts.

Volume 1, which also appeared in 1985, contains an introduction, notes on classification and nomenclature, a key to the families, and covers the Atypidae to Theridiosomatidae.

My criticisms are of the format only and should not obscure Roberts's sparkling achievement in producing a work that many an academic biologist would regard with pride as crowning a lifetime's effort. Yet Roberts is an amateur and still a young man, which makes his success all the more remarkable. But we should be used to the pre-eminence of amateurs in British araneology: Roberts is simply the latest to take his place in an unbroken succession of distinction stretching back to the middle of the last century. And it will be well into the next century before a further major work on the British spider fauna is attempted. Again it will be produced by an amateur, not least because, by then, taxonomic studies may well be considered an improper activity for a university or museum biologist. □

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• *Creative Intelligences*, edited by Richard L. Gregory and Pauline K. Marstrand, presents papers from the British Association Meeting in 1986. Animal and manufactured cognition are covered, from the development of intelligence through species to studies of intelligent machines. Newly published by Frances Pinter, London/Ablex, New Jersey, the book costs £20, \$29.50.

Ways to get into the solid state

C.J. Gilmore

Solid State Chemistry: Techniques. Edited by A.K. Cheetham and Peter Day. *Clarendon*: 1987. Pp. 398. £35, \$70.

SOLID state science has been in the headlines recently with the advent of a new class of metal oxide superconductors capable of operating at temperatures much higher than hitherto possible. A whole new world in science and technology has opened up, and it was an appropriate time for the appearance of a book devoted to the techniques of solid state chemistry — the very methods used by research groups exploring the world of high-temperature superconductors. It is intended for final-year undergraduates and first-year graduate students working in solid state chemistry, and a companion volume surveying compounds with important and useful properties is scheduled for publication soon.

There are ten chapters, each covering a technique or related set of techniques in detail, and each written by a separate specialist. Often the result of this method of putting together a textbook is a piecemeal product, but in this case the strong editorial hand of Cheetham and Day has succeeded in producing a book of remarkable homogeneity. There is, of course, some diversity of writing style and nomenclature from chapter to chapter, but the overall uniformity is very good, and the book also achieves the right balance of theory and description for its intended readership. Only the index is rather weak, with a mere four pages of entries. In a book such as this, where cross-references from chapter to chapter are necessarily minimal, a more detailed index would have added greatly to the ease of use.

The techniques discussed are synthesis, diffraction, X-ray photoelectron spectroscopy, magnetic measurements, optical methods, magic-angle scattering NMR, computational techniques, transport methods, vibrational spectroscopy and thermodynamics. The chapter on computational techniques is a pleasant surprise in an undergraduate text. The use of computers to simulate crystal structures and their physical and defect properties, and hence the ability to carry out on a computer experiments that are impossible in a laboratory, is a technique of increasing importance (although one by no means universally accepted by the scientific community). However, as its use grows, and as methods become increasingly sophisticated, a future generation of superconducting materials may be

designed by computer simulation studies.

Most of the book is equally up to date, with the references complete to 1985. The chapter on diffraction, for example, discusses synchrotron X-ray and neutron spallation sources which, by virtue of their intensity and wavelength tunability, are opening new doors in the crystallography of powders, and, at the other end of the complexity spectrum, biological macromolecules. The chapter also includes a section on electron microscopy, something of such importance in solid state chemistry, however, that it really deserved

a chapter to itself — all too often the topic appears as an adjunct to a chapter on X-ray crystallography.

This is not a book that one person can review — its scope is too wide — so I have discussed it with several colleagues, who all share my enthusiasm. The field is not overloaded with general texts, so the book should find a wide audience, and I look forward to the second volume in due course. □

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Thinking very big

Owen Gingerich

Cosmic Understanding: Philosophy and Science of the Universe. By Milton K. Munitz. *Princeton University Press*: 1987. Pp.287. \$25, £15.80.

FOR those who live with complete faith in the power of reason and who believe that the observable cosmos is all there is or was or ever will be, Munitz's *Cosmic Understanding* should be a profoundly disturbing book. In a thoughtful and closely structured account, Munitz defends two intertwined concepts. First, reality, often depicted solely as a simple physical process, albeit wondrous and infinite, is actually something mysteriously deeper. In particular, he argues that the evolutionary nature of the universe points to some larger transcendental quality. Secondly, he reasons that the perceptual world is a human construct, always only incompletely knowable.

Is reality limited to a physically describable universe? Munitz reaches back to ancient Greece to show that great philosophers have often thought otherwise. Anaximander's transcendental Boundless, an inexhaustible, eternal source without shape, quality or form, gives birth to the mortal cosmos. Heraclitus's Logos, like the Delphic oracle, "neither declares nor conceals but gives a sign" to the enlightened about the unity of nature that transcends the cosmos itself. Parmenides's Being, Munitz suggests, is not some particular entity that *exists*, but rather the idea *that* the world exists.

After this selective tour through the pre-Socratics, Munitz turns to the question of the intelligibility of the universe itself. He mentions first the problem addressed in Kant's epistemology, that the known world must conform to the mind's own structuring capabilities, and then poses the question of the meaning of language itself as analysed in Wittgenstein's later work.

Having laid the foundations for a challenge to the commonly held view that our

theories are describing physical reality as it "really" is, Munitz moves, seemingly effortlessly, from philosophy to modern cosmology and physics. He describes, in a clear fashion, such technical points as Robinson-Walker space time, the Penrose-Hawking theorems, and the inflationary model of the expanding universe. These lead to a discussion of a series of ever-expanding conceptual horizons. All of this is grist for his neo-Kantian mill, which, to my mind, effectively pulverizes the traditional realistic metaphysical views. He writes:

The only access [to an intelligible universe] is via a cosmological model. The universe as a whole can be described only by means of the grammar of the cosmological model. . . . The grammar is not external to that entity; it is internal to it. It constitutes what it is to be such-and-such an intelligible universe. Therefore, apart from that grammar (that model), there is no intelligible universe, no universe as a whole.

Munitz now comes to the apex of his argument, that the universe is embedded into some larger, unknowable whole without time, space, or dimension. Most of us are familiar with the concept of transcendence from theism, where the transcendent is identified with God, the Supreme Being who is wholly different from the spatio-temporal world of objects He created. Munitz pays homage to this Western tradition with an extended critique of Spinoza's rationalistic realism and Spinoza's God, so dear to Einstein's heart. From this discussion there emerges the idea of an "existent", a class containing at least one, non-fictional, temporally bound entity. Since modern cosmology has converged to a view in which the universe has a beginning and ending, the universe, Munitz claims, qualifies as an existent.

If his argument has an Achilles' heel, it may lie here, for much of modern cosmology reckons with a semi-infinite universe, one with a beginning, but without a distinct ending. Despite his insistence that a temporal duration is required for an "existent" and despite extensive technical discussion of the beginning of the universe, Munitz glosses over its ending.

Without fully facing whether the universe will have a temporal end, Munitz goes on to argue that the universe is not just an existent: it is a Boundless Existent. Here the second major theme of the book comes into focus: that the universe can be known only incompletely. I recall a physicist friend arguing that if humanity could survive long enough, we would eventually learn all there is to know about nature, science would put itself out of business, and humankind would be "reduced to analyzing the plots of Japanese novels." Munitz's view (and mine) is entirely different. He writes:

There is consequently no reason to believe that the series of descriptions of the known universe will have a final stage (however long cosmological inquiry may be continued), a stage in which a perfect and complete model will have been realized that exhibits no bounds, poses no problems, and satisfactorily answers all questions. If by 'boundless,' in one of its senses, is meant 'endless,' then cosmological inquiry is fated to be boundless, since the means for carrying on such inquiry — the use of cosmological models — will always be conceptually bound.

In a final chapter Munitz makes clear that he is by no means setting up a philosophical case for theism based on modern evolutionary cosmology. His argument is only that "there is a dimension of Reality that is beyond all actual or possible conceptual analysis and rational comprehension". Munitz's God, if there be one, is unknowable; Boundless Existence tells us that our personal lives, our death, and the fate of mankind as a whole "are bathed in a sea of no meaning, no intelligibility, no hope, no fear".

As a theist in the familiar Western tradition, I find Munitz's arguments both challenging and unsatisfying. He makes a strong case against the simple material realism of much popular modern science in which divine reality supposedly has no place. Yet, just as he poises on the brink of finding meaning in the transcendental nature of the universe, he retreats to a "serenity of purposelessness". Silence is enforced by the utter impossibility of saying anything informative about Boundless Existence, he says; thus, by stating that the universe is without purpose, he has overstepped the bounds of his own logic. In any event, he goes on to assert that enlightened awareness of this transcendental quality can come through some sort of mystical experience. Just when I expected him to bring on the saints or gurus, the book ended. Nevertheless, he has argued brilliantly for the depth and thickness of reality, and as a distinguished theologian recently wrote to me, "I am sure that within that thickness and depth is religious reality, God". □

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Charting a course for the future

Sarah Tyacke

The History of Cartography. Vol. 1: Cartography in Prehistoric, Ancient, and Medieval Europe and the Mediterranean. Edited by J.B. Harley and David Woodward. University of Chicago Press: 1987. Pp.599. £78.95, \$100.

THE history of cartography is not an old subject, but a relatively new one coinciding with the rise in self-awareness of cartography itself as a subject at the end of the nineteenth century. The present volume seeks to synthesize past scholarly work and present research on the cartographic or map-like endeavours of those societies which flourished around the Mediterranean and in the rest of Europe from the prehistoric period to about AD 1500.

This is an ambitious task, one which earlier writers on the history of cartography have normally eschewed on the grounds, as the geographer Max Eckert put it in 1925, that "too much of the groundwork is lacking".

There comes a time, however, when a subject needs to mature, to survey itself, to consider its weaknesses and strengths; this volume demonstrates that historians of cartography have reached that point. It will be the standard reference work on the subject and, even more importantly, by its revelation of lacunae in both our knowledge and interpretation will provide a platform for future research. Much of this research will have to be collaborative and international. The further five volumes of the project, which will take us into the twentieth century, and will include the history of non-European societies in Asia, are expected to follow the same open-ended pattern.

To praise a volume for demonstrating what is lacking may seem churlish at first sight. But indications of what still needs to be researched can be made only by contributors who are able to perceive some sort of historical pattern in their material.

Thus various general points emerge from the chronological chapters: for example, that prehistoric 'map-making' and modern indigenous or native map-making have been all too readily treated as totally interdependent; the prehistoric has often been divorced from its chronological stratum and the implication, from what little evidence there is, that the map-like drawings might have been an act of religious ritual rather than a record of space, has been missed.

Another contributor, O.A.W. Dilke, points out that the lack of a careful critical edition of the surviving manuscripts of Ptolemy's *Geography* remains an impediment



Far-flung places — the Cynocephali (top right), a race of dog-headed people thought to be associated with Islam, featured on didactic mappaemundi as prime targets for Christian missionaries. Detail taken from the Borgia map of c. 1430.

ment to our understanding of Graeco-Roman mapping — this in spite of the illuminating chapters on Greek and Roman geography by Dilke in the present book. His succinct account of the Romans' practical large-scale cadastral surveys, which were not equalled until the eighteenth and nineteenth centuries in Europe, is excellent.

It is also made clear that in the mediaeval period large areas of Europe were not mapped, but we still do not have union lists of all the surviving local and regional maps which would perhaps modify our views. The chapter on the origin and compilation of portolan charts (nautical charts of the Mediterranean sea) reveals that it was these instruments which were most widely sold and used in Europe, and that the chart constitutes the one major area where a new type of map was introduced in the period 1300–1500.

In general, the volume is spared what one commentator Youssouf Kamal, in the context of the origins of portolans, described as "hallucinations scientific", to which historians of such early periods of cartography are prone. The volume successfully places the map as artefact in the context of what the society concerned is assumed to have regarded as its uses — religious, geographical, administrative, cadastral, navigational — and does away with the monopoly of the interpretation of maps as merely 'scientific' objects. The gaps, the connections between mapping traditions and other questions may remain, but they can at least be formulated more easily within this well-researched and documented framework.

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The best for British superconductivity?

David Caplin

Eleven British universities are busy finalizing bids to host the first University Research Centre in high-temperature superconductivity. But there are questions to be asked about the establishment of such a Centre, and about the squeezing of further funds for superconductivity from other areas of science.

OVER the past few weeks, many of Britain's high-temperature superconductivity researchers have had to spend more energy on deciding how to respond to the Science and Engineering Research Council's (SERC) latest initiatives than on science. At the end of July, SERC sent out invitations to eleven institutions to bid for the very first University Research Centre (URC) in high-temperature superconductivity, with responses requested by 15 September (see *Nature* 328, 370; 1987). A couple of weeks later, there came the formal announcement of the £2-million that has been set aside by SERC for research grants in superconductivity *outside* the proposed URC.

All this sounds like good news for the British effort in high-temperature superconductivity, which so far has been notable for its quality, but not for its quantity. SERC seems to be doing its best, but there are serious problems. First of all, it seems that neither the money for the URC, nor that for the research grants, is new, but has come from squeezing SERC's already hard-pressed budget even further. This robbing Peter in order to launch Paul goes against the whole spirit of the recent Advisory Board for the Research Councils' (ABRC) discussion document *A strategy for the science base* (see *Nature* 328, 280; 1987), which recommended strongly the establishment of interdisciplinary URCs. They made it clear that, to be effective, the proposed Centres must be adequately resourced, and that that will require substantial *additional* funds.

Research assistants

Secondly, the Centres are each to have 20 to 30 post-doctoral research assistants — where are they to come from? There are simply not the skilled people available who are willing to accept the relatively low pay, and the lack of any kind of career structure. Because of the political significance that has been attached to the URCs, perhaps a way will be found to make these posts more attractive, but then the inevitable result will be that it will become even more difficult than it is now to fill such post-doctoral positions elsewhere.

So, although it is clearly SERC's present intention to have a dual-track approach to high-temperature superconductivity, with one URC now (and

perhaps a second one later), and also a substantial research grant programme spread over several other universities, the limitations on both money and manpower may make this impossible. Grants may be awarded, but if the hands are not available — and it is hands, not big machines, that are needed most urgently — the grants will be useless. Those in other research areas who have had to give up substantial funding will soon be asking for some of their money back. But there will also be ideological pressures. After all, it is being claimed that British university research is too dispersed and on too small a scale to be competitive, and that Centres are the way forward. The politicians may well ask why SERC is trying to maintain both the URC and the research grant approaches, and force a choice. It will not be easy to ditch a URC whose launch has been proclaimed as the answer to our problems.

Will a URC for high-temperature superconductivity actually be useful? The ABRC report identified some of the features it thought warranted a Centre. In its multidisciplinary aspect, high-temperature superconductivity fits the bill to perfection. People who had never before talked scientifically to each other now collaborate closely. Ceramicists, chemists, crystallographers, physicists, electrical engineers are all in it together. It is tempting to construct a Noah's Ark, to recruit a couple of each species and give them the money to get on with the job.

Unfortunately, high-temperature superconductivity, which is still some months away from its first birthday, doesn't work quite like that. When some reasonably convincing physical explanation for the superconducting mechanism is found, it will provide a signpost for the direction of a flood of new work. Again, the techniques for making the materials are still being tried out, and for the technologically important thin films there is no way yet of knowing whether, for example, molecular beam epitaxy or chemical vapour deposition or some other technique will be the most useful. Consequently, specifying now what should be in the high-temperature superconductivity URC is fraught with uncertainty.

What about the senior scientific staff? There are groups of scientists, such as the inorganic chemists, who have just met

superconductivity for the first time in their professional lives, and whose skills are essential for the new materials. Are they going to immediately uproot themselves to move to a new Centre? Over time, and as the subject develops, priorities will change and people move. But it cannot happen overnight.

Strategy

Certainly, British researchers in superconductivity recognize that there has to be an overall strategy, and that interdisciplinary and inter-institutional collaborations are essential. Some degree of concentration of resources is appropriate, but I have yet to hear anybody, academic or industrialist, put a scientific case for focusing efforts at this early stage on a single URC in high-temperature superconductivity. Indeed, the institution that receives it may well be handicapped for months, as it struggles to organize the Centre while others get on with the science.

But there is a positive side to the URC initiative: even if they had not already done so, the process of formulating a bid for a multidisciplinary Centre has forced people together across departmental boundaries. It is perhaps a fair criticism of some institutions that those boundaries are often marked by substantial barriers, not because of any lack of good will, but because it requires conscious effort and precious time to overcome them.

It is clear that many of the eleven institutions that have been asked to bid for the URC in high-temperature superconductivity will be able to put forward a strong and coherent multidisciplinary programme, but there is no one outstanding candidate. Not only that, but university superconductivity groups in Britain, both large and small, have worked together informally over the past few months with great success. Will SERC be able to find a way of exploiting the strong spirit of co-operation that is in the air? Or will it be forced to select a single Centre, with the 'winner' uncertain of whether it is fortunate in having been chosen, the losers perhaps dispirited, and a hiatus for the British contribution to high-temperature superconductivity? □

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Comet showers as a cause of mass extinctions

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If at least some mass extinctions are caused by impacts, why do they extend over intervals of one to three million years and have a partly stepwise character? The solution may be provided by multiple cometary impacts. Astronomical, geological and palaeontological evidence is consistent with a causal connection between comet showers, clusters of impact events and stepwise mass extinctions, but it is too early to tell how pervasive this relationship may be.

BRIEF intervals of greatly increased impact frequency must have occurred on Earth during occasional comet showers. Such showers are caused by perturbations of the Oort comet cloud due to close passages of neighbouring stars. Here we explore the hypothesis that these comet showers may have caused stepwise mass extinctions through multiple impacts within a short period of time. This hypothesis is based on calculations of orbital dynamics, age data on terrestrial impact craters, and the stratigraphic record of impact and extinction events. We do not maintain that this hypothesis has been demonstrated to be correct, only that there is enough evidence that it must be taken seriously and should be carefully tested.

Impacts of Earth-crossing asteroids take place randomly in time¹. A significant fraction of comets, however, should arrive in discrete showers triggered by a relatively close passage of a star or interstellar gas cloud². Early calculations^{2,3} indicated that such comet showers should last ~1 Myr. We present a detailed calculation of the temporal profile of such a comet shower, which turns out to last ~3 megayears, with the bulk of the comets arriving within ~1 Myr.

A number of geological and palaeontological observations suggest that such showers have occurred: clustered crater ages, stratigraphic horizons of impact ejecta closely spaced in time, and evidence for three stepwise mass extinctions spanning intervals of 1–3 Myr during the past 100 Myr, corresponding to the Eocene/Oligocene (E/O), Cretaceous/Tertiary (K/T) and Cenomanian/Turonian (C/T) boundary zones. This tentative evidence warrants further investigation.

In 1980, Alvarez *et al.*⁴ presented evidence that the end-Cretaceous mass extinction was caused by a large impact. Abundant supporting evidence comes from studies of the Cretaceous/Tertiary boundary focusing on iridium content^{5,6}, osmium isotopes⁷, the chemistry and mineralogy of the boundary clay⁸, spherules of possible impact-melt origin^{9–11}, shocked mineral grains^{12–14}, soot particles¹⁵, and the mechanism needed to distribute boundary materials worldwide¹⁶. Alternative viewpoints^{17–22} have been debated in the literature^{23,24}. The late Eocene mass extinction (~36–40 Myr BP) also displays evidence for one or more impacts^{25–38}.

Some palaeontologists have argued that the end-Cretaceous mass extinction took place gradually over a few million years³⁹, but the relevant fossil record is complex^{24,40}. Dinosaur fossils are too rare to determine whether their extinction was abrupt or gradual. Some invertebrates (such as ammonites and rudists) were seriously depleted 1–2 Myr before the end of the Cretaceous. Other invertebrates (bivalves, brachiopods, bryozoa and foraminifera) and the coccoliths were decimated abruptly,

during an interval of evolutionary diversification, at precisely the time of the K/T impact.

Fischer and Arthur⁴¹ first suggested that mass extinctions might occur periodically; Raup and Sepkoski⁴² presented quantitative palaeontological support for a 26-Myr extinction periodicity. To explain periodic mass extinctions, two of which roughly coincided with major impacts, a mechanism for producing periodic impacts was needed. Hills² showed that disturbances of the Oort cloud could produce comet showers and clustered impacts, and suggested a relation between this mechanism and the end-Cretaceous mass extinction. Independently, Davis *et al.*⁴³ and Whitmire and Jackson⁴⁴ proposed periodic disturbances of the Oort cloud by a distant companion star of the Sun, and Rampino and Stothers⁴⁵ suggested that the disturbances result from oscillations of the Solar System through the galactic plane.

The evidence for periodicity was hotly debated, but a realization emerged that non-periodic comet showers resulting from encounters with passing stars should also be considered, as in Hills' original suggestion. Muller^{3,46} first noted that a mass extinction occurring as the result of multiple impacts during a comet shower might take place in discrete extinction steps and thus be confused with a gradual extinction. Meanwhile, the concept of stepwise mass extinctions was independently developed from high-resolution stratigraphic studies—by Kauffman^{47–49} for the Cretaceous/Tertiary and Cenomanian/Turonian mass extinctions, by Keller^{32–34} for the late Eocene extinctions, and by Raup⁵⁰, who used the taxonomic data base of Raup and Sepkoski^{42,51}. Evidence for multiple impacts in the Late Eocene^{45,52}, and hints of clustering in time of impact crater ages⁵³, strengthened the case for comet showers. Somewhat later, another scheme for explaining periodic comet showers was proposed, based on an as yet unobserved Planet X⁵⁴, but this model seemed to be inconsistent⁵⁵.

Our purpose here is partly to review the literature bearing on the hypothesized relationship between comet showers and stepwise mass extinctions, and partly to summarize the results of our investigations of this hypothesis during the past two years.

Astronomical theory of comet showers

Although the original impact explanation for the iridium anomaly at the K/T boundary term was termed "the asteroid-impact hypothesis", it was realized that a comet impact would provide an equally plausible explanation for the observed iridium excess. It is not easy to decide which explanation is more likely, as the non-volatile component of comets probable has a chemical composition close to that of the primitive car-

bonaceous chondrites⁵⁶. As comets probably consist of a roughly equal mixture of silicates, hydrocarbons and volatile ices, their expected bulk iridium content is somewhat less than one-third that of an undifferentiated carbonaceous asteroid. Another difference is that long-period comets typically strike the Earth at about three times the velocity of asteroids, and therefore impart an order of magnitude more energy than an asteroid of comparable mass.

There is little specific evidence to distinguish between a cometary or an asteroidal impactor at the K/T boundary. Palme⁵⁷ has argued that the siderophile signature in the boundary clay "does not match... the pattern of unfractionated meteorites (chondrites)," and an iron-asteroid impactor has been suggested. Asaro⁵⁸, on the other hand, considers that the elemental ratios in the boundary clay do match CI carbonaceous chondrites.

Asteroids are expected to collide with the Earth at essentially random intervals, with an expected half-life for Earth-crossing asteroids of ~ 30 Myr (ref. 1). Some Earth-crossing asteroids may be derived from short-period comets, so their population and flux may be modulated by comet showers. We might expect each comet shower to be followed by an extended tail of impacts by asteroids derived from short-period comets. But these (possibly overlapping) tails may be difficult to discriminate from the steady-state flux of long-period comets and Earth-crossing asteroids. In the calculations of the temporal profile of a comet shower we can safely neglect the small contribution of comets-turned-asteroids, at least for the major part of the shower.

Comets are also expected to collide with the Earth at random times, arriving from the outer Oort cloud (from distances of $> 2 \times 10^4$ astronomical units (AU)), where the perturbations by the galactic tides⁵⁹ and the frequent perturbations by passing stars provide a trickle of new comets into the planetary region. On rare occasions, the passage of a star close to the Sun, or a close passage by a massive interstellar gas cloud, will significantly perturb the inner Oort cloud as well. This region is expected to contain a much higher density of comets than the outer Oort cloud (refs 2, 60, 61 and M. Duncan, T. Quinn and S. Tremaine, preprint). Any perturbation of this inner region that fills the loss cone will cause a comet shower to occur throughout the inner planetary region, within one orbital period of the comets in the inner Oort cloud (ref. 62 and J. Heisler, S. Tremaine and C. Alcock, preprint).

On entering the inner planetary system, the orbital semi-major axes (and thus the orbital energies) of the comets are strongly perturbed by Jupiter and Saturn, so that most are removed or 'lost' from the Oort cloud (hence the names 'loss cone' or 'loss cylinder' for the region in velocity space within which the orbits pass through the inner planetary system). Weissman⁶³⁻⁶⁴ showed that a typical Oort-cloud comet (semi-major axis 2.5×10^4 AU) passing within the orbit of Jupiter would either be ejected from the Solar System on a hyperbolic orbit or captured to a shorter-period orbit where stellar perturbations were not important, with $< 5\%$ of the comets returning to the outer Oort cloud. Weissman found that the average long-period comet made only five perihelion passages, with an average time of 6×10^5 yr between the first and last passage. Of the long-period comets, 65% were ejected, 27% randomly disrupted and 8% lost to a variety of lesser end-states, such as sublimation of all volatiles or perturbation to a Sun impacting orbit. A very small fraction of the long-period comets, on the order of 4×10^{-4} , evolved to short-period orbits (periods < 200 yr) after an average of almost 400 returns. The short-period comets return frequently to the planetary region until they are ejected by perturbations caused by Jupiter (on a timescale of ~ 1 Myr), totally disintegrate (after several thousand returns) or evolve both physically and dynamically to asteroidal objects in Earth-approaching orbits.

Comets perturbed into the planetary region in a comet shower from the inner Oort cloud would be expected to behave similarly to the long-period comets described above, except for the effect of their small initial orbital semi-major axes. We have studied

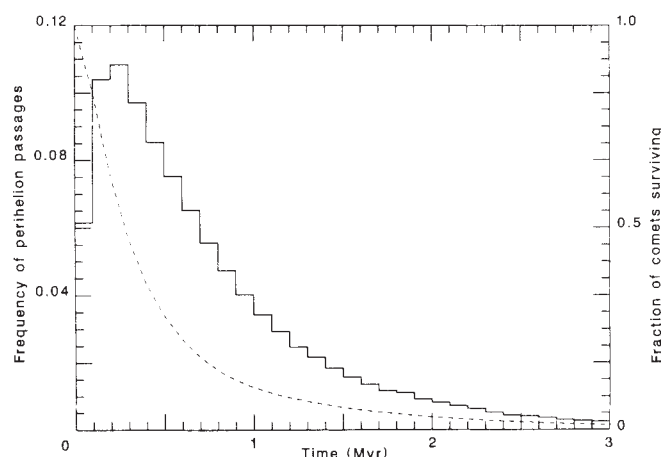


Fig. 1 Profile of a comet shower caused by an instantaneous perturbation. The dashed line shows the rapidly falling fraction of surviving comets; that is, comets that are neither being thrown out of the inner Oort cloud by planetary perturbations, nor are destroyed by physical mechanisms such as break-up or sublimation. The solid line indicates the frequency of perihelion passages, which measures the probability of an impact on Earth.

the evolution of a cometary shower in time using Weissman's^{63,64} Monte Carlo simulation model for the long-period comets. In doing so we have assumed that comets in the inner and outer Oort cloud are physically similar, in that their sublimation lifetime, probability of random disruption, and propensity for forming non-volatile crusts are the same. In our simulation we started 5×10^6 comets in orbits with orbital energies uniformly spread between semi-major axes of 3×10^3 and 10^4 AU, perihelia uniformly spread between 0.01 and 4.0 AU, and initial orbital phases spread uniformly in mean anomaly. It was found that the average Earth-crossing comet made 8.6 returns to the planetary region, with an average lifetime of 0.48 Myr. The largest fraction of comets, 63%, were ejected by Jupiter perturbations, 31% were lost to random disruption and 5% had their perihelia perturbed out of the planetary region to orbits in the outer Oort cloud.

Figure 1 shows the number of comets surviving in the system with time (dashed line). The number initially declines rapidly, with a half-life of < 0.3 Myr. Also shown (solid line) is the number of perihelion passages versus time. This curve rises quickly as comets begin arriving in the planetary region on their long-period orbits. It declines less rapidly because, although the number of comets is monotonically decreasing, the orbital periods of the surviving comets are also decreasing, leading to more frequent perihelion passages for each survivor. Thus, a small fraction of the comets in the system accounts for a large fraction of the later cometary returns.

The peak of the distribution for the number of perihelion passages versus time (Fig. 1) has a full-width at half-maximum (FWHM) of 0.8 Myr. The times after the initial perturbation for 25, 50 and 75% of the perihelion passages to have occurred are 0.3, 0.5 and 0.9 Myr, respectively. More than 90% of the returns occur in the first 1.8 Myr after the perturbation of the Oort cloud and 99% in 4.0 Myr.

The shapes of the two curves described above are relatively insensitive to the physical and dynamical parameters that enter the Monte Carlo simulation. Variations in the disruption probabilities, sublimation lifetimes or orbital energy distribution in the inner Oort cloud do not change the peak flux or shower duration by more than 20%. This result was confirmed by running the Monte Carlo simulation while varying the model parameters. Weissman's simulation model has already been 'tuned' to mimic the evolution of the observed long-period comets; substantial changes of parameters outside the ranges studied would result in disagreement with observed cometary

behavior. After finishing these calculations, we learned that Fernández and Ip⁶² report similar calculations, consistent with Fig. 1.

If the perturbing force is due to the distant passage of a massive interstellar gas cloud rather than a close stellar encounter, the resulting disturbance will be spread over ~ 1 Myr, and will broaden the distribution of arrival times to a FWHM of ~ 1.5 Myr. A star passing at a distance of 10,000 AU and traveling at 40 km s^{-1} perturbs the inner Oort cloud during an interval of only ~ 0.01 Myr; an interstellar gas cloud (or subcondensation within a larger gas cloud) passing at 10 parsecs with a relative speed of 20 km s^{-1} exerts a significant perturbation during ~ 1 Myr. Alternatively, if the Sun has a companion star which traverses the inner Oort cloud every $\sim 26\text{--}28$ Myr^{43,44,65,66}, the duration of the perturbation can be similarly extended.

It is difficult to assess the exact number of comets in a shower, as that figure will be sensitive to the population of comets in the inner Oort Cloud as well as the velocity, mass and encounter distance of the passing star. Heisler *et al.* (J. Heisler, S. Tremaine and C. Alcock, preprint) have shown that even the flux of comets from the outer Oort cloud can easily vary by a factor of 3–5 owing to random stellar perturbation. Thus, whereas current estimates place comets as accounting for $\sim 50\%$ of the steady-state cratering on the Earth⁶⁷, the true fraction may be considerably different if the current short-term estimates of the cometary flux are different from the true long-term average. Taking current best-guesses for the relevant parameters (but keeping in mind the factor of 3–5 additional uncertainty), we estimate that a major cometary shower involving $\sim 10^9$ comets of >3 km diameter perturbed into Earth-crossing orbits would result in ~ 20 impacts, and would occur every 300–500 Myr. Minor showers involving $\sim 10^8$ comets and ~ 2 such impacts would occur more frequently, every 30–50 Myr.

The conclusions useful for a comparison with possibly related terrestrial geological phenomena are as follows: (1) the time spans of clustering of terrestrial phenomena should be comparable to that shown in Fig. 1; (2) if the distribution roughly corresponds to that in Fig. 1, but is somewhat broader, the cause could be a comet shower involving comets in somewhat more distant orbits in the Oort cloud, or it could indicate a triggering by the passage of a companion star or a massive distant object such as an interstellar gas cloud; (3) if the distribution is spread out over a length of time much larger than that shown in Fig. 1, it is unlikely that a single comet shower can explain the events; (4) similarly, if the distribution of possibly impact-related

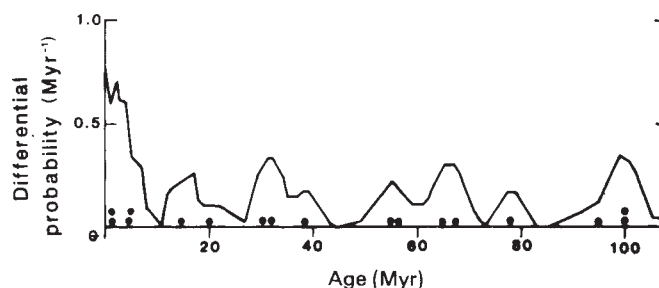


Fig. 2 Smoothed probability distribution of ages of observed terrestrial impact structures during the last 100 Myr, derived from ages presented in ref. 52. The probability distribution has been smoothed by a 6-Myr running mean. Dots represent central values of estimated ages.

phenomena exhibits a significantly shorter duration than that shown in Fig. 1, a comet shower cannot explain the events.

Geological observations of impact events

Seventeen terrestrial impact structures are known with mean diameters of ≥ 5 km and for which the best estimated ages are <100 Myr and are thought to have an uncertainty (1σ) less than ± 15 Myr⁶⁸.

Figure 2 shows a probability distribution of age for these structures, obtained by smoothing with a 6-Myr running mean the probability distribution for each impact structure, represented by a box of width 2σ ⁶⁸. Four peaks rise above the rest in the distribution, one crowded against the present, a second centred near 35 Myr, a third near 65 Myr and a fourth near 99 Myr.

It should be kept in mind that the moderately well dated impact structures are only a small sample of all the impact structures that have probably been formed on the continents in the past 100 Myr. Most distributions of 17 ages drawn at random from a uniformly distributed population of ages would have peaks similar in magnitude or larger than those centred at 35, 65 and 99 Myr. It may be significant, however, that the largest Phanerozoic impact structure so far discovered corresponds to one of the events in the broad peak centred at 35 Myr, and that the age of the impact event recorded by the noble-metal-bearing clay layer at the Cretaceous/Tertiary boundary coincides with the center of the 65 Myr peak.

Table 1 Ages of strewn fields of impact glass*

	Dating method	Age (Myr)	Reference
Indochinites and philippinites (includes microtektites)	⁴⁰ Ar– ³⁹ Ar	$0.690 \pm 0.028^\dagger$	71
	Fission track	0.693 ± 0.025	70
Darwin glass	K–Ar	0.70 ± 0.08	
	Fission track	0.74 ± 0.04	118
Australites	Fission track	0.830 ± 0.028	70
	K–Ar	0.86 ± 0.06	119
	⁴⁰ Ar– ³⁹ Ar	0.887 ± 0.034	71
Irgizites‡	Fission track	1.07 ± 0.06	120
Ivory Coast tektites§ (includes microtektites)	Fission track	1.08 ± 0.10	121
South Australian high-Na tektites	Fission track	$\geq 8.55 \pm 0.90$	122
Moldavites	Fission track	14.7 ± 0.4	121
Libyan Desert glass¶	Fission track	29.4 ± 0.5	121
North American tektites (includes microtektites)	Fission track	34.6 ± 0.7	121
	Stratigraphic	36.0 ± 0.5	75
Clinopyroxene-bearing <u>micro</u> spherules and microtektites in <i>Globorotalia cerroazulensis</i> zone	Stratigraphic	36.4 ± 0.5	75
Cryptocrystalline <u>micro</u> spherules and microtektites in <i>Globigerapsis semiinvoluta</i> zone	Stratigraphic	36.4 ± 0.5	75

* Objects which may once have been glass but are no longer glassy, such as spherules found in the clay at the K–T boundary⁶⁰ are not included.

† Errors quoted are standard deviations.

‡ Associated with Zhamanshin crater, USSR.

§ Associated with Bosumtwi crater, Ghana.

|| Associated with Ries crater, West Germany.

¶ Apparently associated with Oasis crater, Libya (undated).

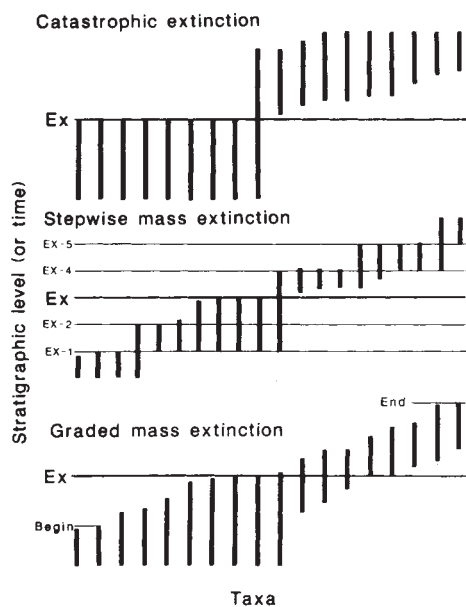


Fig. 3 Models of extinction patterns of taxa (vertical lines) within the framework of the three major theories for mass extinction. 'EX' represents the stratigraphic level (or time) of either maximum taxonomic loss or of the extinction of certain characteristic taxa (such as biostratigraphic indices). EX-1, EX-2, for example, define stepwise extinction events as components of a single mass extinction interval⁴⁹.

A variety of impact-formed glasses constitute additional sources of evidence for the times of impact events (Table 1). In three cases, well dated glasses can be correlated with well dated impact craters, but for the remainder of the cases the ages of the glass represent an independent data set. Two clusters of ages are found for the impact glasses, one near 1 Myr and one centred at ~35 Myr, which fall on the two broad peaks of Cenozoic crater ages.

For many years it was generally believed that the tektites found distributed across Southeast Asia, the Philippines, Indonesia and Australia, as well as microtektites widely distributed in deep-sea deposits in the Indian Ocean, belong to a single strewn field, commonly referred to as the Australasian strewn field⁶⁹. Recently, Storzer and Wagnet⁷⁰ presented new high-precision fission-track ages which suggest that the tektites found in Australia (australites) may be ~0.14 Myr older than the Southeast Asian and Philippine tektites. The apparent difference in the ages has been supported by two new ⁴⁰Ar-³⁹Ar ages⁷¹. If this difference is real, the Australasian tektites comprise two major strewn fields that may be partly overlapping. However, a careful search in deep-sea sediments has revealed only one clear-cut layer in the stratigraphic distribution of the microtektites⁷². Two other strewn fields of impact glass, one of which includes the Ivory Coast microtektites, are ~0.2 Myr older than the australites.

Of particular interest is the close spacing of ages of three strewn fields of impact glass in the late Eocene. Two closely spaced layers of microspherules are found in deep-sea sediments in the late Eocene *Globorotalia cerroazulensis* foraminiferal zone. The microspherules in the uppermost layer are crystal-free siliceous microtektites, which are closely similar in chemical and isotopic composition to the North American tektites^{25,26}. The tektites and correlated microtektites are distributed over part of North America, the Gulf of Mexico, the Caribbean region and the western North Atlantic²⁶⁻²⁹. Cryptocrystalline and crystal-bearing glass spherules more basic in composition than the microtektites occur in a layer a few tens of centimetres below the North American microtektite horizon at several sites

in the Gulf of Mexico and the Caribbean²⁶. These spherules are also widely distributed in the central Pacific, where they were originally miscorrelated with the North American microtektites²⁷⁻³¹. A strong iridium anomaly, with a peak iridium abundance of up to 4.1 p.p.b.^{35,36} is coincident with this spherule layer^{26-29,37}. In Barbados, the stratigraphic position of the clinopyroxene-bearing spherule layer is marked by a moderate iridium anomaly^{27-29,38}.

Another well-defined late Eocene, microspherule layer has been found in the western Pacific at DSDP (Deep Sea Drilling Program) Site 292 in core 38-2, between 75 and 80 cm depth (see Fig. 5), and possibly in the Indian Ocean at DSDP Site 216²⁷⁻²⁹. This layer occurs in the *Globigerapsis semiinvoluta* zone^{27-29,32-34}, and has been correlated previously with one or the other of the microspherule layers in the *G. cerroazulensis* zone^{26,37}; however, the biostratigraphy demonstrates that it is older than the microspherule layers in the *G. cerroazulensis* zone.

In summary, four weak peaks are observed in the impact crater age distribution in the past 100 Myr; two clusters of ages of impact glass are broadly coincident with the crater-age peaks in the Cenozoic. Represented within the clusters are major strewn fields of glass and microspherules, three of late Eocene age and at least two and possibly three of Quaternary age. The ages of the Quaternary strewn fields of tektites and microtektites are distributed over ~0.4 Myr. The exact time separation of the late Eocene impact glass horizons is not known; for plausible sedimentation rates, deposition of the two most closely spaced glass horizons was probably separated by between ten and a few tens of thousands of years. Deposition of these glass horizons is separated, in turn, by ~0.5-1.0 Myr from the lower glass horizon. The clustering of ages of most of the known glass strewn fields into two compact age groups is unlikely to be the result of events that are randomly distributed in time⁶⁸. A burst or shower of impacts in the late Eocene seems to be indicated, and a burst may have occurred in the Quaternary.

Palaeontological observations of extinctions

A mass extinction annihilates 50-95% of ecologically and genetically diverse lower taxa within 1-3.5 Myr in well-dated extinction events for which closely spaced stratigraphic and taxonomic data exist (C/T, K/T and E/O events)⁴⁹. Published taxonomic and biostratigraphic data support this concept⁴²: the 7 Myr average interval of ref. 42 is based on taxonomic data time-averaged to the stage level.

Figure 3 illustrates the three major concepts of mass extinction⁴⁹. In graded mass extinctions, extinction rates are higher than background levels, but taxa disappear randomly throughout this interval, possibly along an ecological gradient from initially stenotopic to more eurytopic groups. This pattern is attributed to accelerated intervals of environmental change resulting from terrestrial causes (such as eustasy or climate), producing global ecological crises. Catastrophic mass extinction theory⁴ views global annihilation among genetically and ecologically diverse organisms as occurring near-simultaneously (days to centuries) as a direct or indirect result of enormous perturbations of the global environment usually attributed to extraterrestrial causes (such as impact or radiation). Stepwise mass extinction^{32-34,49} occurs as a series of discrete steps of highly accelerated or catastrophic extinction spread over 0.5-3.5 Myr intervals, each affecting only a portion of the global biota. Individual extinction steps may also be characterized, even predominantly, by ecological shock to surviving species manifested in abrupt population decline and/or restriction to refugia habitats; the latter reappear in recovery faunas as 'Lazarus taxa'⁷³. In the well documented K/T, C/T and E/O events, extinction steps are ecologically graded from (first) stenotopic organisms (tropical reef biotas), proceeding through progressively more tolerant groups, to a final extinction of ecological generalists.

Proof of these hypotheses requires detailed (centimetre-scale) global stratigraphic analyses of extinction patterns among

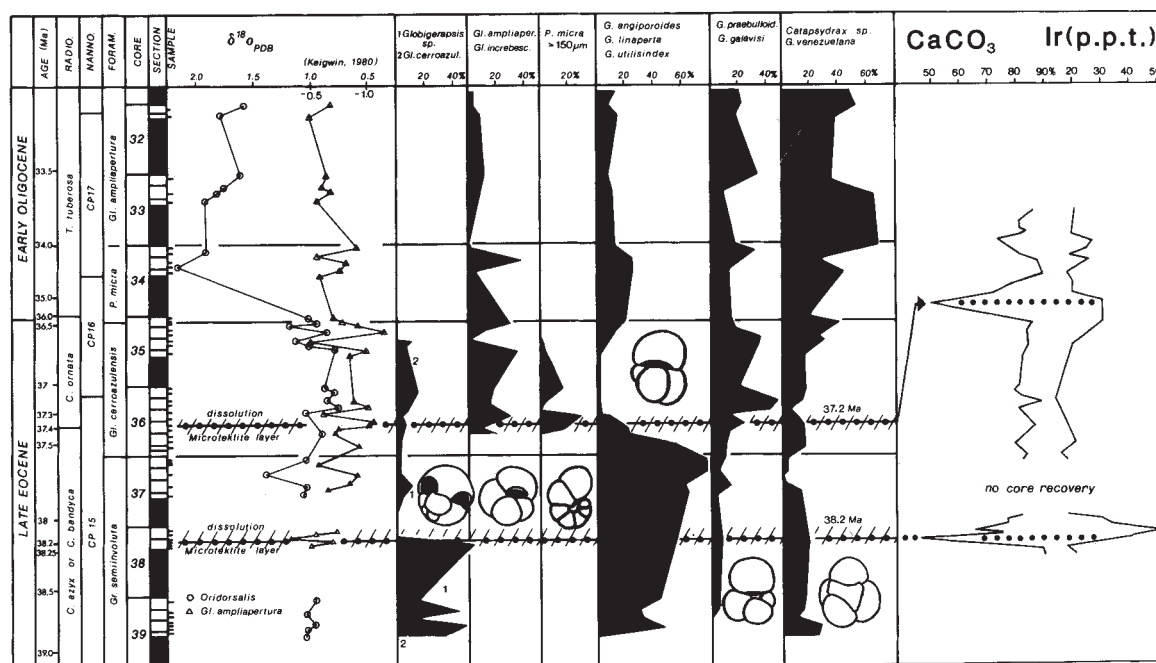


Fig. 5 Microtektite layers, per cent CaCO_3 , iridium, oxygen isotope data and species abundance changes during the late Eocene to early Oligocene in the western equatorial Pacific DSDP Site 292.

partly due to global cooling or decreased productivity, and/or acid rain due to major impact events^{76,77}, but it is very difficult to separate the effects of a productivity change from those of dissolution; further studies are necessary to elucidate this relationship. Middle to late Eocene stepwise extinctions result from multiple causes, as there is no evidence of impacts associated with the two steps preceding or that following the deposition of the presently known impact glass layers.

Stepwise extinctions of molluscs. Gulf Coast molluscan data compiled from the field and literature show extinction patterns across the Eocene/Oligocene boundary that also support the concept of a stepwise mass extinction. Molluscan radiation in the middle Eocene reached its peak of species diversification in the Cook Mountain Formation (384 gastropod and bivalve species) and the overlying Gosport Formation (385 species), and then declined dramatically through a series of three discrete extinction events, two of them major, which occur between the middle/late Eocene and the Eocene/Oligocene boundaries.

The initial extinction event occurred just below the middle/late Eocene boundary, near the stratigraphic boundary between the Gosport and the Moody's Branch formations. Species extinctions across this boundary were 89% for gastropods (233 of 263 species) and 84% for bivalves (102 of 122 species). This extinction event corresponds to step 2 (Fig. 4) of the planktonic foraminiferal extinctions discussed above. A second step accelerated molluscan extinction took place near the top of the Moody's Branch Formation and equivalent strata. This extinction event lies within the early late Eocene (within planktonic foraminiferal zone P15; *Globigeropsis seminvoluta* biozone). Species extinctions across this boundary were 72% for gastropods (139 of 193 species) and 63% for bivalves (50 of 80 species). This extinction event may be associated with step 3 (Fig. 4) of the planktonic foraminiferal extinctions observed in deep-sea sediments. The final extinction event occurred at the Eocene/Oligocene boundary among already depleted Gulf Coast molluscan faunas, with a loss of 58 of 60 gastropod species (97%) and 23 of 26 bivalve species (89%). Most of these species disappeared in the upper half of the Yazoo Formation, but the exact stratigraphic location of the extinction has not yet been determined. The extinction took place within 1 Myr or less.

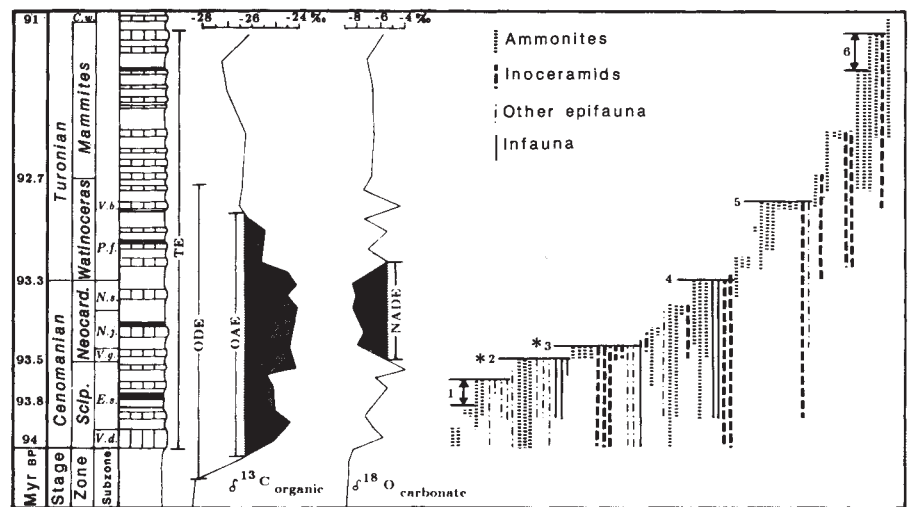
Among mollusca, bivalves and planktonic foraminifera, warm-water genera suffered a higher specific extinction rate than cool-tolerant forms in the late Eocene/Oligocene boundary stepwise mass extinctions; in the middle late Eocene stepwise mass extinctions this was true for planktonic foraminifera. But macroinvertebrates show no clearly defined trends for the terminal Gosport event other than the greater effect on Gastropoda than on Bivalvia and the indiscriminate extinction of a broad range of genetic and ecological groups (see ref. 78 for a more detailed account).

Cretaceous/Tertiary boundary. Because of erosion or non-deposition due to sea-level lowering during the Cretaceous/Tertiary boundary interval, few sections preserve a record of mass extinction among shallow-water invertebrates during this time⁴⁷. Most marine macrofaunal data are from Danish and Spanish sequences in shelf chalk facies^{40,49,79-81}. Data from siliciclastic shelf facies are available from Texas⁸², Mexico and Brazil^{47,83}. Tethyan carbonate platform sequences in Jamaica^{47,49,81,85}, Puerto Rico, the Spanish Pyrenees^{47,86,87} and the Middle East document collapse of the Cretaceous reef ecosystem below the K/T boundary. A composite analysis of the data suggests that one or more extinction steps preceded the main K/T extinction event.

The most prominent precursory extinction event occurred within the lower part of the Upper Maastrichtian *Abathomphalus mayaroensis* zone and follows Lower Maastrichtian diversification of Cretaceous marine biotas⁴⁹. This event decimated specialized ammonites^{47,81,88,81}, diverse suspension-feeding deep-water benthos⁴⁷ and most tropical stenotopic reef-forming groups^{47,49,85-87}. The extinction step is most dramatically seen in the tropical Caribbean data⁸⁴, where all 24 genera and 83 species of rudistids (reef-building bivalves) that were present near the Lower/Upper Maastrichtian boundary disappeared with the lower *A. mayaroensis* zone, even though mollusc-rich shallow carbonate platform environments persisted into the Palaeocene. A similar extinction pattern is suggested in Spanish and Middle Eastern data^{47,86,87}. Only a single rudistid specimen, belonging to a eurytopic temperate-tropical group, is known at the K/T boundary (in Denmark)⁹⁰.

In addition to this clearly documented first step, there are

Fig. 6 Composite plot of the ranges of molluscan species in the western interior of the United States having last occurrences in the Cenomanian-Turonian boundary interval. The data are integrated from numerous sections, calibrated to individual volcanic ash (ben-tonite) and Milankovitch climate cycle strata, and are plotted against the western interior standard reference section (left) at Rock Canyon anticline near Pueblo, Colorado¹⁰³. The $\delta^{13}\text{C}_{\text{organic}}$ and $\delta^{18}\text{O}_{\text{carbonate}}$ values (relative to PDB) are from the same section¹⁰⁶. Species ranges are a graphic composite from stratigraphic sections analysed by Elder^{103,104}, and from range data of Koch^{113,114}, Cobban and Scott¹¹⁵ and Cobban¹²³. Biostratigraphic zones and subzones, in ascending order, are: the *Vascoceras diartianum* (V.d.) and *Euomphaloceras septemseriatum* (E.s.) subzones of the *Sciponoceras gracile* biozone (Scip.); the *Vascoceras gamai* (V.g.), *Neocardioceras juddi* (N.j.) and *Nigericeras scotti* (N.s.) subzones of the *Neocardioceras juddi* biozone (Neocard.); the *Pseudospidoceras flexuosum* (P.f.) and *Vascoceras birchbyi* (V.b.) subzones of the *Watinoceras* biozone; the *Mammites nodosoides* biozone; and the *Collignoniceras woollgari* (C.w.) biozone. Numbered horizontal lines or intervals mark the possible stepwise extinctions; those marked by an asterisk denote major, well-documented extinction steps recognizable at most localities. Approximately 15 additional, mostly infaunal taxa (not plotted) are known to range through the entire interval, and many other species are either too rare or their taxonomy is too poorly known to determine their ranges accurately.



preliminary suggestions that a second and third extinction step may have preceded the end of the Maastrichtian. The possible second step⁴⁹ is in the middle of the *Abathomphalus mayaroensis* biozone, and its principal victims were the last generalized framework-building rudistids (Radiolitidae, Hippuritidae), characteristic inoceramid bivalves, generalized deep-water benthic macroinvertebrates and mid-water ammonite lineages^{47,81,88,91}. The possible third precursory step, only 0.05–0.1 Myr before the main boundary extinction, is suggested by a short-term decline in specialized calcareous plankton^{92–97}, losses among deep-water shelled benthos, and extinction of the last giant solitary rudistids (*Titanosarcolithes*) and the remaining ammonites (Denmark excepted)⁹¹.

The biggest extinction step occurred at the Cretaceous/Tertiary boundary, and is marked by the near-simultaneous global extinction of most calcareous and siliceous oceanic plankton^{92–96}, diverse brachiopods^{40,98}, bryozoans^{79,99} and molluscs⁹⁰, and terminal loss of 'endangered species' among already-decimated, typical Cretaceous groups (such as rudists, ammonites and inoceramid bivalves). This extinction event is associated with the large iridium anomaly, shocked mineral grains and other evidence for impact at the K/T boundary⁴⁰. A 0.05 Myr interval characterized by small, depauperate, ecologically generalized basal Palaeocene biotas followed this end-Cretaceous extinction; this was succeeded within stabilizing marine environments (refs 81, 92–96, 100 and Fig. 1 of ref. 101) by rapid radiation among new and surviving Lower Palaeocene taxa and a return of 'Lazarus taxa'⁷³ from as yet undiscovered refugia.

Published plots of data from Denmark^{40,49,98,99} suggest several more extinction steps among Lower Palaeocene bryozoa⁹⁹ and brachiopod species^{40,98} up to 0.25 Myr above the K/T boundary, but these apparent extinction steps seem to coincide closely with the tops of samples spaced ≥ 1 m apart. We conclude that the sampling of the Danish Palaeocene is not yet sufficiently detailed to determine whether further extinction steps occur above the Cretaceous/Tertiary boundary.

Thus, one strong extinction step precedes the Cretaceous/Tertiary boundary, and others are suggested by existing data. More detailed (centimetre-scale) studies of the best available sections will be required in order to test whether other steps occurred. **Cenomanian/Turonian boundary.** The Cenomanian/Turonian (C/T) mass extinction occurred near a high-stand of global eustatic sea level and broad amelioration of global climates. Four extraordinary environmental perturbations account for the

unusual magnitude of the marine faunal extinction (30% of genera⁴² and >70% of species)^{47,103,104,113–117} in the C/T boundary interval (see Fig. 6): (1) the Bonarelli oceanic anoxic event (OAE)¹⁰, represented worldwide by organic-rich, laminated shales, bituminous limestones^{47,103,104}, or by widespread deep-sea carbonate dissolution; (2) the lowering of average oceanic water temperatures¹⁰⁵, coupled with a rapid multi-stage temperature rise in shallow temperate seas (TE)^{105–107}, thus altering marine thermal regimes and enhancing density stratification; (3) a desalination event, lasting ~0.2 Myr, in the shallow, normal-marine, epicontinental seas of North America (NADE)¹⁰⁶ near peak eustatic high-stand, implying regionally extensive rainfall; and (4) a 1.5-Myr-long interval of massive destabilization of the global climate and ocean systems (ODE), indicated by abnormally rapid (50–100 kyr) large-scale fluctuations in the stable isotopes of carbon and oxygen^{106,108,109}. The unusual rate, magnitude and character of these environmental perturbations may be impact-induced, but the evidence is inconclusive. A modest iridium anomaly just below the Cenomanian/Turonian boundary is suggested from North American and European data (C. J. Orth and F. Asaro, personal communication), but needs further verification. Two major impact craters (Steen River and Logoisk) have isotopic ages with uncertainties that encompass the Cenomanian/Turonian boundary interval^{52,110}.

The C/T mass extinction occurred over a 2.5 Myr interval (Fig. 6), and is characterized by brief intervals of highly accelerated extinction separated by longer recovery intervals with lower background extinction rates^{47,103,104,107}. Two major molluscan extinction steps are globally recognizable in the C/T extinction interval (events 2, 4; Fig. 6) and are widely defined by the extinction of common taxa at many sections in North America. Four other extinction steps may also be present (events 1, 3, 5, 6; Fig. 6; but these are based on composite ranges of less common and more geographically restricted taxa. Following a buildup of subtropical-warm temperature diversity during the late Cenomanian rise in sea level, the initiation of the Bonarelli OAE, a global shift in ^{13}C abundance¹⁰⁶ and early phases of oceanic temperature decline (coupled with rapid temperature increases in temperate epicontinental seas^{105,107}) were associated with event 1 (Fig. 6), in which a major loss of keeled planktonic foraminifera (*Rotalipora* lineage)^{111,112} and a modest molluscan extinction occurred within 0.05–0.1 Myr^{103,104}.

The first major C/T molluscan extinction (event 2, Fig. 6) occurred within a 0.05 Myr interval at the top of the *S. gracile*

biozone (93.5 Myr), when accelerated thermal and chemical fluctuations in the marine realm (Fig. 6) and initiation of a large reduction in marine salinity in the North American Seaway ~ 0.3 Myr below the C/T boundary (NADE, Fig. 6) caused a 15–30% loss of genera in the central interior seaway^{103,104,107,111} and a cumulative loss of 78–80% of molluscan species for extinction steps 1 and 2^{113–115}. A brief oxygenation and re-colonization event followed in warm epicontinental seas during the Bonarelli OAE and was ended by environmental perturbations associated with renewed regional oxygen depletion at event 3 (Fig. 6)^{47,103,104}.

A few surviving generalized molluscan and foraminiferal lineages persisted through the peak Bonarelli OAE before becoming abruptly extinct at the second major extinction (event 4, Fig. 6), coincident with peak excursions of the $\delta^{18}\text{O}$ and $\sigma^{13}\text{C}$ curves (Fig. 6)¹⁰⁶. This extinction event marks the biostratigraphically defined C/T boundary¹¹⁶, and is equivalent to a nannofossil biozone boundary in the western interior of North America (ref. 117). Following a 50-kyr-long Lower Turonian anoxic interval (latest Bonarelli OAE) with virtually no benthic fauna, diverse warm-water biotas were repeatedly re-established, and then rapidly annihilated at events 5 and 6 (Fig. 6). The upper of these events coincided with the initiation of rapid global eustatic sea-level fall and the cooling of epicontinental seas, and was coupled with the last big stable-isotope excursions before the establishment of normal temperature marine conditions (Fig. 6).

Thus, several discrete extinction steps span the C/T boundary and appear to have been associated with extraordinary climatic, temperature and oceanic perturbations. These oscillations, acting on a global biota narrowly adapted to warm, equable, maritime-dominated climates, appear to be the direct cause of stepwise extinction; they may have been driven by extraterrestrial forcing.

Conclusions

We have presented three independent pieces of evidence supporting a connection between comet showers and clustering in terrestrial cratering and mass extinctions.

From the astronomical side, observations of comets have led to the theory of a comet cloud (the Oort cloud) surrounding the planetary system at a large distance from the sun; we calculate the temporal profile of a comet shower triggered by a star passing through the Oort cloud. From the geological side, four weak peaks are found in the age of distribution of impact craters over the past 100 Myr, as well as two compact clusters of ages of impact glass broadly coincident with crater-age peaks. From the palaeontological side, recent observations indicate a stepwise character for some well-documented mass extinctions in the past 100 Myr which roughly coincide with three of the four peaks in crater ages and which have a duration compatible with comet shower predictions.

It is too early to predict whether the hypotheses of comet showers will offer a general and global explanation for any or all mass extinctions. We enteratin a spectrum of differing opinions on this question, but the evidence cited here is suggestive of a causal connection in at least some cases.

Definitive testing of this hypothesis will require a new level of detailed inquiry in each of the relevant fields. From orbital dynamics we need a deeper understanding of the frequency and intensity of comet showers and of the pattern of impacts to be expected from the range of possible disturbances of the Oort cloud and from non-shower, random events. From impact geology we need a more complete catalogue of terrestrial craters and more refined dating of these structures. A useful test, the dating of a large sample of lunar craters, must unfortunately wait for the indefinite future. Physical and chemical stratigraphers must search for evidence of impact (iridium, tektites and microspherules, shocked mineral grains) at 1–10-cm intervals throughout large portions of the stratigraphic record. Bio-

stratigraphers must document at very high resolution the fine structure of mass extinction intervals, a task complicated by the necessity, in many cases, of dealing with the statistics of small samples. Ocean and climate modelling will eventually be necessary to understand the effects of multiple impacts on the biosphere. The intriguing results obtained to date suggest that this will be a worthwhile enterprise.

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ARTICLES

Stratospheric trace gases in the spring 1986 Antarctic atmosphere

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The atmospheric absorption features of over 500 infrared solar spectra recorded at McMurdo Station, have been analysed to determine the vertical column abundances of trace gases crucial to the understanding of the 'ozone hole' phenomenon.

SATELLITE measurements^{1,2} have now established that the springtime ozone depletion, first reported by Farman *et al.*³, extends throughout the polar vortex, a region of the atmosphere isolated by the polar circulation during the Antarctic winter and usually centred over eastern Antarctica. During September and October 1986, a period during which we monitored the atmosphere above McMurdo Station (78° S, 166° E), we were fortunate that we were near the centre of the ozone hole on two occasions; 15-22 September and 15-23 October. The edge of the hole, as defined by the maximum ozone gradient, was close to McMurdo on three occasions: 5-8 September, 23-28 September and 6-13 October. Thus, our observations sampled the polar atmosphere deep inside the vortex as well as the warmer regions close to the edge.

The observations were made with the JPL MkIV Interferometer, a high-resolution Michelson interferometer built specifically for recording atmospheric spectra from remote ground-based sites, aircraft and from stratospheric balloons. The instrument is double-passed with one fixed and one moving corner reflector, allowing a 200-cm maximum optical path difference. For the majority of the spectra recorded at McMurdo the maximum path difference of the scans was limited to 66 cm, giving an unapodized spectral resolution of 0.01 cm⁻¹. The carriage which holds the moving reflector is driven by a flexible

integrating nut riding on a lead screw. This arrangement, together with the double-passed optical scheme, makes the instrument resistant to the effects of mechanical distortion and shock; this was especially important when operating under the conditions of wind and thermal stress encountered in the Antarctic environment. The spectral range of the instrument is covered by two detectors: an InSb detector is used for the shorter wavelengths (1.8-5.5 μm, 1,800-5,500 cm⁻¹) and a HgCdTe photoconductor for the range 5.5-15 μm (650-1,800 cm⁻¹). Both detectors are cooled to liquid nitrogen temperatures. The optical path difference between the two arms of the interferometer is monitored during the scan by a He-Ne laser whose interference fringes are used to trigger the sampling of the two signal channels. At the sampling rate of 10⁴ s⁻¹, each pair of one million-point interferograms is recorded in 100 s.

An advantage of this technique is that the measurements of all of the constituents are made simultaneously in the same airmass, so that the results presented here represent self-contained inventories for each day. Due to the low solar angles, the stratospheric airmass sampled by our instrument was, at times, as much as 200 km away from the observation site, a point to remember when comparisons are made between these and other measurements made in slightly different airmasses.

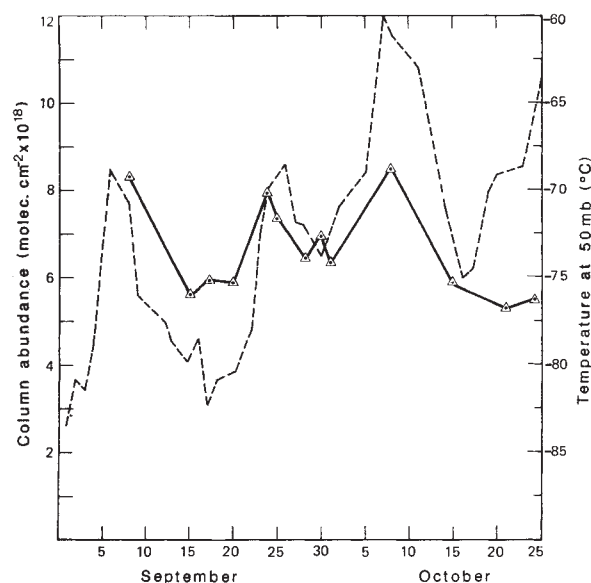


Fig. 1 Vertical column abundances of ozone over McMurdo Station during September and October, 1986. The dotted line shows the temperature at the 50-mb level.

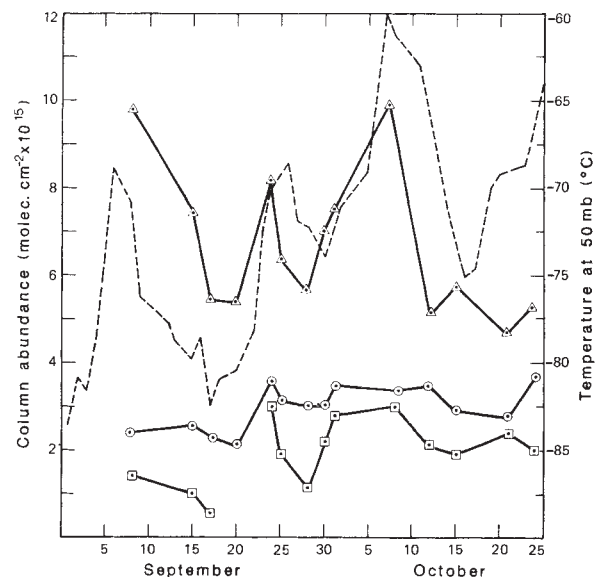


Fig. 2 As Fig. 1, for HNO_3 (Δ), NO ($\circ$) and NO_2 ($\square$). Note that the HNO_3 values are shown at half scale.

Data from 14 days have been analysed to examine the variations of the column abundances of several related gases whose behaviour should provide clues for discriminating between the different mechanisms that have been suggested to explain the ozone hole. Table 1 lists the values of the solar zenith and azimuth angles for the beginning and end of the observation periods during which the spectra were recorded. The results for the stratospheric trace gases are summarized in Table 2 and in Figs 1, 2 and 3. The short-term variations (on a timescale of days) are a reflection of the movement of the polar vortex; the longer-term changes reveal the variations of stratospheric composition in the vortex that accompany the ozone depletion. In common with other studies, we have chosen the temperature at the 50-mb level as the characteristic parameter to describe the sampled airmass and against which to examine correlations of the column abundances of the related trace constituents. These temperatures were collated from radiosonde measurements made at McMurdo by the United States Navy and by the University of Wyoming (D. Hoffman, personal communication) during our observing period, and are superimposed on Figs 1, 2 and 3 showing the time variation of the constituent abundances.

The analysis of the spectra was performed by scaling an

assumed volume mixing ratio profile for a particular molecule until the spectrum calculated using this profile best matched the measured spectrum over a small wavenumber interval, or 'micro-window', in which the spectral lines of the molecule are free from blending with lines of other gases. Table 3 lists the micro-windows used for the molecules reported here. The temperature and pressure profiles used in the calculation were interpolated from the radiosonde measurements of Hoffman *et al.*⁴; as these radiosonde flights were made about twice per week, the potentially large temperature error on some days dictated our choice of temperature-insensitive spectral lines whenever possible.

The retrieval of a vertical column abundance from an atmospheric absorption feature requires knowledge of both the volume mixing ratio profile of the gas and the trajectory of the solar radiation through the atmosphere. The latter was established by numerical tracing of a ray backwards through the refracting atmosphere until it emerged at the correct astronomical solar zenith angle, computed from the known latitude, longitude, time and the solar ephemeris. The accuracy of the parameters used in this calculation becomes critical for low solar-elevation observations; but a sensitive internal check on the validity of the ray geometry assigned to each spectrum is provided by CO_2 , whose line strengths and mixing ratio have been determined to

Table 1 Summary of observation periods used for the present analysis

Date	Local time*	Solar angle (deg.)		Local time	Solar angle (deg.)	
		zenith	azimuth		zenith	azimuth
8 September	11.23	84.6	22.0	11.57	84.1	13.5
15 September	12.09	81.3	9.9	13.00	81.1	357.1
17 September	11.15	81.3	23.2	11.43	80.8	16.2
20 September	13.46	79.5	345.2	13.58	79.7	342.2
24 September	11.37	78.1	17.1	11.41	78.1	16.1
25 September	13.13	77.3	353.0	13.40	77.5	346.2
28 September	13.05	76.1	354.7	13.41	76.4	345.7
30 September	13.46	75.7	344.3	14.04	76.0	339.8
1 October	13.23	75.0	350.0	14.12	75.8	337.7
8 October	05.47	87.3	103.5	06.14	85.9	96.7
12 October	14.00	71.4	339.9	14.13	71.7	336.7
15 October	05.02	86.9	114.3	05.48	84.6	102.8
21 October	14.02	68.2	339.0	14.30	68.8	332.0
24 October	13.46	66.8	342.8	14.06	67.2	337.8

* Local time, GMT + 12 h.

Table 2 Observed vertical column abundances (molec. cm⁻²)

Molecule	O ₃ [†] (×10 ¹⁸)	O ₃ (×10 ¹⁸)	NO (×10 ¹⁵)	NO ₂ (×10 ¹⁵)	HNO ₃ (×10 ¹⁵)	ClONO ₂ (×10 ¹⁵)	HCl (×10 ¹⁵)
Date							
8 September	7.79	8.47 ± 0.20‡	2.4 ± 0.5	1.4 ± 0.5	19.6 ± 0.5	2.5 ± 0.5	1.56 ± 0.10
15 September	6.15	5.57 ± 0.20	2.6 ± 0.6	1.0 ± 0.4	14.9 ± 0.4	1.8 ± 0.3	1.48 ± 0.10
17 September	5.51	5.98 ± 0.15	2.3 ± 0.4	0.5 ± 0.5	10.9 ± 0.2	1.5 ± 0.2	1.58 ± 0.10
20 September	5.83	5.92 ± 0.05	2.1 ± 1.0	2.0 ± 4.0	10.8 ± 0.2	2.0 ± 0.2	2.04 ± 0.15
24 September	8.04	8.06 ± 0.20	3.6 ± 0.6	3.0 ± 2.5	16.6 ± 0.5	4.5 ± 0.4	2.69 ± 0.15
25 September	7.04	7.37 ± 0.15	3.1 ± 0.2	1.9 ± 0.4	12.7 ± 0.4	3.7 ± 0.2	2.84 ± 0.10
28 September	6.26	6.44 ± 0.10	3.0 ± 0.2	1.1 ± 0.4	11.2 ± 0.2	3.0 ± 0.2	2.85 ± 0.05
30 September	6.48	6.99 ± 0.15	3.0 ± 0.5	2.2 ± 0.5	14.1 ± 0.2	3.8 ± 0.4	2.88 ± 0.10
1 October	6.26	6.33 ± 0.12	3.5 ± 0.5	2.8 ± 0.5	15.1 ± 0.2	3.7 ± 0.2	2.99 ± 0.10
8 October	7.28	8.55 ± 0.05	3.4 ± 0.3	3.0 ± 0.2	20.0 ± 0.2	3.5 ± 0.1	4.15 ± 0.05
12 October	6.56	6.95 ± 0.05	3.5 ± 0.4	2.1 ± 0.5	10.2 ± 0.1	3.4 ± 0.5	4.20 ± 0.05
15 October	5.78	5.85 ± 0.05	2.9 ± 0.2	1.9 ± 0.3	11.5 ± 0.2	2.5 ± 0.1	4.17 ± 0.05
21 October	5.13	5.29 ± 0.60	2.8 ± 0.8	2.4 ± 0.6	9.4 ± 0.4	2.4 ± 1.2	3.65 ± 0.25
24 October	5.67	5.55 ± 0.40	3.7 ± 0.5	2.0 ± 1.5	10.7 ± 0.3	1.5 ± 0.8	5.29 ± 0.25

* HF, $1.80 \pm 0.01 \times 10^{15}$. † Latitude-corrected TOMS O₃ column values, see text. ‡ The global errors represent precisions, see text.

high accuracy elsewhere. Tests showed that for weak, temperature-independent absorption features, and solar zenith angles $\leq 85^\circ$, the error due to the use of an incorrect volume mixing ratio profile is small. The analysis was therefore concentrated on the spectra taken at the highest elevation angles, even though these, because of their characteristically shallower absorptions, resulted in somewhat lower precision. Of the molecules discussed here, HNO₃ shows the largest dependence of the derived column abundance on the assumed vertical profile. This results from two factors: (1) the accessible spectral lines are temperature-dependent (-0.4% per $^\circ\text{C}$) and (2) they are opaque at the line centres, resulting in nonlinear growth of their absorption. Numerical tests using a range of realistic vertical profiles showed that the derived HNO₃ column varies over a maximum range of $\pm 15\%$. But provided the shape of the profile did not change appreciably during the observation period, errors in the assumed profile introduce a systematic bias which does not invalidate the derived day-to-day variations in the column amount.

The absolute accuracy of the molecular spectral parameters⁵ used in the analysis limits the accuracies of the derived column abundances for most of the gases discussed here. The exceptions to this are NO and NO₂, which have such small absorptions that on days when the SNR is poor the accuracy of the column becomes limited by random error. The ClONO₂ Q-branch at 780 cm^{-1} has only recently been measured at low temperatures⁶ and over an extended spectral range⁷; the results of these new measurements indicate a potential 30% error in our derived column values for ClONO₂. Spectral line parameters of the ν_5 band of HNO₃ used here are incomplete; Brown *et al.*⁵ claim an accuracy of 10% for the integrated intensities of the four fundamental bands of HNO₃. As the microwindow used for HNO₃ includes five manifolds, each of which contains several prominent lines, we believe that 20% is an appropriate accuracy for our derived columns, although individual spectral lines may be uncertain by more than this. The O₃, HCl and HF line

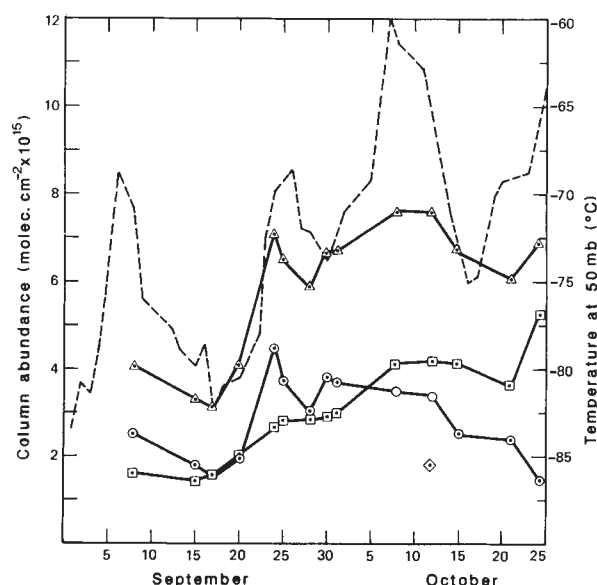


Fig. 3 As Fig. 1, for the chlorine reservoir species HCl (□), ClONO₂ (○) and their sum (Δ). The column abundance value for HF obtained on 12 October 1986 is also shown (◇).

parameters are estimated to be accurate to 5% (ref. 5). But errors in the assumed strengths of the spectral lines enter the analysis as systematic effects so that, provided the same features are used for each spectrum analysed, only errors in the assumed temperature dependences of the line strengths and Lorentz widths coupled to actual temperature changes will affect the precision of the day-to-day variations. These temperature dependences are well known in all cases (except for ClONO₂) and are automatically accounted for in the analysis procedure. Therefore, as the importance of the derived column abundances lies primarily in their trends and correlations, rather than their absolute values, we feel it appropriate to quote errors corresponding to estimates of relative precision rather than absolute accuracy, so that significant day-to-day variations are still apparent. These error estimates listed in Table 2, are the standard deviations of column abundances obtained from individual spectra. It is evident from examination of the errors that the quality of the data varied considerably, chiefly because of the effects of wind buffeting the instrument on some days.

Trace gases

Ozone. As an example of the precision attainable by this method, Fig. 4 shows measured and calculated spectra at $1,154\text{ cm}^{-1}$, one

Table 3 Principal microwindows used for analysis

Constituent	Central frequency and width (cm ⁻¹)
CO ₂	2,632.36, 2,636.63 (0.2)
O ₃	1,154.61 (0.2)
HNO ₃	868.10 (2.0)
ClONO ₂	780.25 (0.7)
NO	1,900.05, 1,903.13 (0.2)
NO ₂	2,914.65 (0.1)
HCl	2,843.63, 2,925.90, 2,963.29 (0.1)
HF	4,038.96 (0.2)

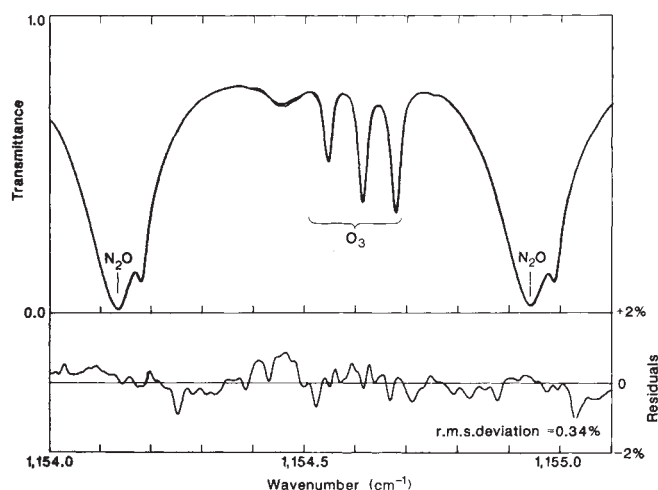


Fig. 4 Comparison of calculated and measured spectra over a region including the triplet of ozone lines at $1,154.6 \text{ cm}^{-1}$ used to derive the O_3 column abundances. The observed data were taken on 8 October 1986 at a solar zenith angle 85.9° . The residuals (measured minus calculated) have been included to emphasize the small differences between the spectra.

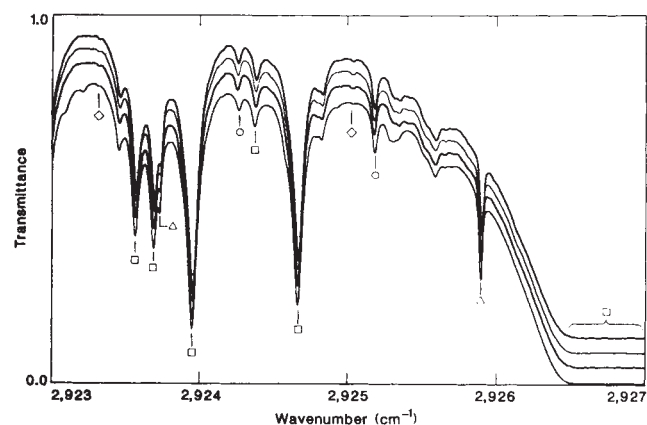


Fig. 5 Examples of a calculated spectrum (lower trace) and three consecutive measured spectra (offset for clarity) taken on 8 October 1986 at a solar zenith angle of 87.1° . These illustrate the reproducibility of the observations and the high quality of the spectral line parameters used in the calculations. Some of the more significant absorptions are identified: $\square$, CH_4 ; $\circ$, H_2O ; $\triangle$, HCl ; $\diamond$, NO_2 .

of many ozone microwindows; the r.m.s. deviation between the calculated and measured spectra is typically 0.35% for a single scan. To validate our ozone measurements, we calculated where the path of the observed solar radiation had passed through the ozone layer and then estimated the total ozone mapping spectrometer (TOMS) O_3 column at that location from the latitudinal O_3 gradient (ref. 2 and A. J. Krueger, personal communication). When we were close to the edge of the ozone hole this gradient was as large as 10% per degree (latitude), and the corrections on these occasions were considerable. Comparison of our O_3 total column values with the latitudinally corrected TOMS values (Table 2) gives a good indication of the general agreement between the methods. The residual systematic difference of $\sim 5\%$ is a reflection of the accuracy of the spectral parameters used in the analysis.

Nitrogen species. It has been postulated⁸ that the Antarctic ozone depletion is a natural phenomenon linked to the solar cycle, the ozone reduction being caused by enhanced levels of nitrogen oxides produced as a result of increased solar activity. Our measured NO_2 values (Fig. 2) were abnormally small, especially in mid-September when they fell below $1 \times 10^{15} \text{ cm}^{-2}$. Bearing

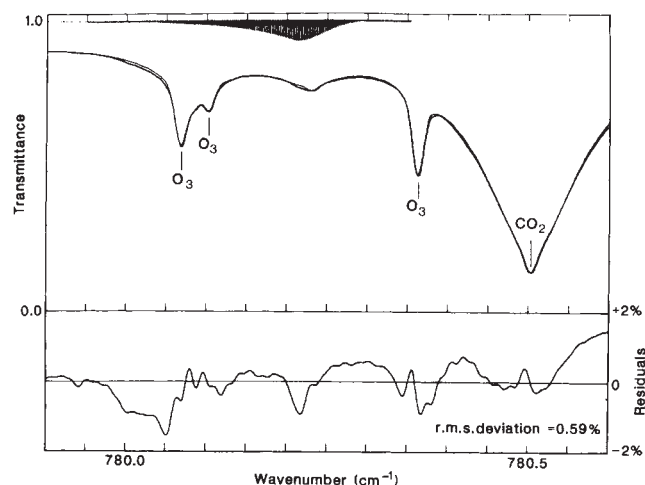


Fig. 6 Observed and computed spectra in the 780.2 cm^{-1} micro-window for ClONO_2 , from data taken on 15 October 1986 at a solar zenith angle of 85.6° . The lower trace shows the residuals while the upper tick marks represent the positions and strengths of the ClONO_2 spectral lines used in the calculation.

in mind the lower altitude of the polar NO_2 layer¹¹, the peak volume mixing ratios were therefore greatly reduced in comparison with mid-latitudes^{9,10}. NO_2 columns recovered to $\sim 3 \times 10^{15} \text{ cm}^{-2}$ when the edge of the hole was close to McMurdo, but never exceeded mid-latitude values. These results agree with the visible measurements of Mount *et al.*¹¹ to within the error estimates. NO followed the same trends as NO_2 , with the difference that the bulk of the NO which, on the basis of mid-latitude profiles, is assumed to be located above the region of the ozone depletion, moderated the fluctuations in its total column abundance. The column abundances of HNO_3 , which are plotted at half-scale in Fig. 2, show large fluctuations, the maximum values being larger by a factor of two than the values obtained from measurements using the same spectral interval at lower latitudes^{9,10}.

Halogen sinks and reservoirs. The behaviour of the principal chlorine reservoirs, hydrochloric acid (HCl) and chlorine nitrate (ClONO_2), is shown in Fig. 3; examples of the spectral fits obtained in the analysis of these gases are illustrated in Figs 5 and 6. The HCl column amounts, which are derived from the analysis of several absorption lines, show an increase from an initial low value of $1.5 \times 10^{15} \text{ cm}^{-2}$ to $4.2 \times 10^{15} \text{ cm}^{-2}$ by mid-October. A further rapid increase to $5.3 \times 10^{15} \text{ cm}^{-2}$ occurred during the last few days of observation, an almost fourfold increase overall. The corresponding column value for the stratospheric component of HCl at mid-latitudes is $1.5 \times 10^{15} \text{ cm}^{-2}$ (refs 9, 12).

The ClONO_2 results show a similar trend (but with much larger day-to-day variations) until the beginning of October, after which there is a marked reversal and the column amounts decrease for the remainder of the period. The polar ClONO_2 column values are considerably higher, with respect to mid-latitude amounts¹³, than the HCl values. The variation of the total amount of chlorine in the reservoirs (which is assumed to be the sum of HCl and ClONO_2) is also plotted in Fig. 3; this shows a very close correlation with the stratospheric temperature and an increase of a factor of two between September and October.

When discussing the sinks and reservoir species, note that the measurements themselves yield values only for the vapour phase abundances of the constituents. The increase in the chlorine reservoirs between September and October, both inside and 'outside' the hole, suggests either that substantial fractions of the HCl and ClONO_2 are held in some condensed form, for example, as polar stratospheric clouds, before October, or that

'missing' chlorine is present in the atmosphere in some other molecular form which we have not yet been able to identify in the spectra. The former possibility is supported, in a qualitative sense, by the correlation of the reservoir species with the stratospheric temperature. In the absence of significant condensation, however, the deficit to be made up by an alternate molecular form of chlorine is large—at least $4 \times 10^{15} \text{ cm}^{-2}$ —and species with even the weakest line strengths would be readily detected at the high signal-to-noise ratio of these observations, unless their absorption bands coincided with opaque regions of the spectrum; strong methane and nitrous oxide lines, for example, hamper the search for Cl_2O_2 in the $1,200 \text{ cm}^{-1}$ region of the spectrum. Conservative estimates of the minimum column amounts of ClO which would give rise to discernible spectral features at 850 cm^{-1} in our spectra in September and October were $2 \times 10^{14} \text{ cm}^{-2}$ and $1 \times 10^{15} \text{ cm}^{-2}$, respectively. The present results taken alone, therefore, do not preclude the possibility that relatively large amounts of ClO, but $< 2 \times 10^{15} \text{ cm}^{-2}$, are present in the stratosphere during September and early October. The missing chlorine cannot be accounted for by ClO alone, however.

In either of the above cases, the partitioning of chlorine between ClONO_2 and HCl is unusual, and very different from the observed situation at lower latitudes, where measurements and model predictions are in good agreement¹³. The mid-latitude column values for ClONO_2 and stratospheric HCl are 7×10^{14} and $1.5 \times 10^{15} \text{ cm}^{-2}$, respectively. By comparison, the corresponding values for McMurdo in October were both approximately $4 \times 10^{15} \text{ cm}^{-2}$. The partitioning between the two reservoirs reverts to a ratio more typical of lower latitudes towards the end of October. It is evident that the chlorine chemistry of the Antarctic stratosphere is perturbed during the early spring season.

The sink for fluorine, hydrofluoric acid (HF), was measured on one day only, towards the end of the observing period at McMurdo, because the wavelength of the absorption band of HF lies outside the normal operating range of the instrument. The spectra that were obtained for HF were of very high quality and the derived column abundance, $1.80 \times 10^{15} \text{ cm}^{-2}$, has an estimated accuracy of 5%. This measurement provides a key reference point in assessing the validity of the Antarctic halogen budget suggested by these results. Manmade chemicals are the only known source of fluorine in the atmosphere and HF in the stratosphere is the direct result of the photolysis of CFCs. The column abundance of HF at McMurdo is about three times larger than the corresponding value at mid-latitudes^{9,12}, presumably a consequence of the stratospheric circulation and the long lifetime of HF. The ratio HF/HCl in the upper stratosphere is important in understanding the effects of injection of halogenated chemicals as it resolves the complication that arises because chlorine has natural as well as anthropogenic sources, coupled with the fact that HCl is removed more easily than HF from the atmosphere. This ratio at mid-latitudes is presently 1/4 (ref. 9), a value which has increased from 1/10 over the ten years since its distribution was first measured¹⁴. From the McMurdo measurements, the fluorine to chlorine ratio for the Antarctic stratosphere was found to be close to 1/4 also, after the total chlorine (taken here as the sum of ClONO_2 and HCl) had reached its maximum value (by the beginning of October). This suggests that by October most of the chlorine was in the

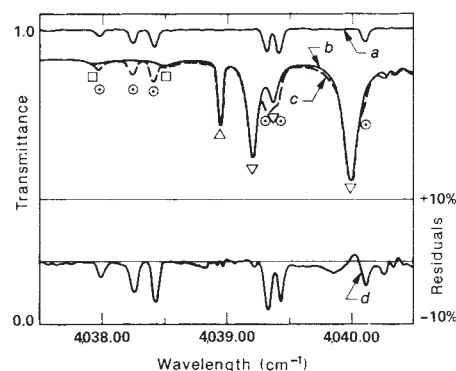


Fig. 7 The spectrum in the region of the R1 line of HF at $4,038.96 \text{ cm}^{-1}$ is illustrated. The upper trace (a), taken from the solar spectrum recorded by the ATMOS instrument¹⁵ from an altitude of 250 km, shows several absorption lines of the 2-0 band of CO in the solar photosphere. The two superimposed spectra are a single scan recorded at McMurdo at a solar zenith angle of 71.5° (b) and the corresponding calculated spectrum (c). The residual plot (d) shows that the large differences between the observed and calculated spectra are due to the solar lines, which are not included in the calculation, and that the HF line is isolated from any interference from either solar or telluric absorptions. ○, solar CO; □, HDO; ▽, H_2O and ◇, HF. The fit to the observed spectrum in the proximity of the HF line is better than 1%.

vapour phase with very little of it being in any form other than HCl or ClONO_2 .

Conclusions

Movement of the vortex caused large positively correlated fluctuations of temperature and all the stratospheric trace gases, making difficult the separation of temporal trends from the effects of spatial inhomogeneity. But, between the two periods when the centre of the vortex was near McMurdo (15–22 September and 15–23 October) even though the temperature increased at all levels there was very little change in the column abundances of HNO_3 , which suggests that if this was indeed the same airmass, little HNO_3 was frozen out.

The chlorine species, on the other hand, show large increases between these two periods, suggesting that during September, a major fraction of the total stratospheric chlorine burden is either frozen out or is present in some molecular form other than HCl or ClONO_2 . The chemical partitioning between HCl and ClONO_2 is also highly abnormal during September, both inside and near the edge of the vortex. While this is not proof of a cause and effect relationship between halogenated source gases and the springtime ozone depletion, it is entirely consistent with such hypotheses.

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The yeast DNA repair gene *RAD6* encodes a ubiquitin-conjugating enzyme

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The RAD6 gene of the yeast Saccharomyces cerevisiae is required for a variety of cellular functions including DNA repair. The discovery that the RAD6 gene product can catalyse the covalent attachment of ubiquitin to other proteins suggests that the multiple functions of the RAD6 protein are mediated by its ubiquitin-conjugating activity.

UBIQUITIN is present in all eukaryotic cells and is one of the most highly conserved proteins known¹⁻⁵. It exists in cells either free or covalently joined to a variety of cytoplasmic, nuclear and integral membrane proteins¹⁻¹⁰. Recent biochemical and genetic evidence indicates that conjugation of ubiquitin to intracellular proteins is essential for their selective degradation¹¹⁻¹⁴. An alternative, non-proteolytic role for ubiquitin, in which its reversible joining to an acceptor protein could modulate protein function, has also been suggested^{2,3,5}, in particular because the ubiquitinated histones H2A and H2B are present in chromatin¹⁵⁻¹⁹.

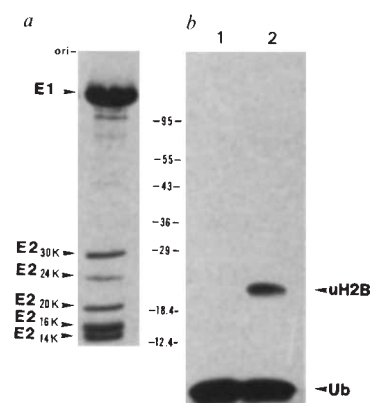
The *RAD6* gene of the yeast *Saccharomyces cerevisiae* is required for a variety of cellular functions including DNA repair, induced mutagenesis, and sporulation^{20,21}. In particular, *rad6* mutants are extremely vulnerable to DNA damaging agents such as ultraviolet light, X-rays and chemical mutagens, and are totally deficient with respect to mutagenesis induced by these agents. The diversity of the phenotypes of *rad6* mutants suggests that the *RAD6* product is central to regulation. We have discovered that the *RAD6* gene encodes an enzyme which can catalyse the covalent attachment of ubiquitin to other proteins.

In vitro, the *RAD6* protein mediates the ubiquitination of histones such as H2A and H2B. The *RAD6* protein is one of several structurally related ubiquitin-conjugating enzymes¹⁻⁴ (also called E2 enzymes) that can be isolated from yeast by ubiquitin affinity chromatography. These enzymes, which display distinct substrate specificities, appear to be highly conserved between yeast and mammals, as is ubiquitin itself. Our results indicate that the multiple functions of the *RAD6* gene product are mediated by its ubiquitin-conjugating activity. They also suggest that ubiquitination of chromosomal proteins by the *RAD6* protein *in vivo* may be responsible for specific alterations in chromatin structure required for DNA repair and for sporulation.

The enzymatic reactions involved in the formation of ubiquitin-protein conjugates have been extensively studied with enzymes isolated from mammalian reticulocytes^{1-4,22}. In an initial, ATP-requiring step, the carboxy-terminal glycine residue of ubiquitin is joined, through a high-energy thiolester bond, to a cysteine residue of the ubiquitin-activating enzyme, E1. The activated ubiquitin is then transferred to specific cysteine residues in several smaller proteins, collectively referred to as

Fig. 1 Components of *S. cerevisiae* ubiquitin-protein ligase system. *a*, Polyacrylamide (18%)-SDS electrophoretic pattern of the DTT eluate from ubiquitin affinity column. Coomassie-stained bands corresponding to the ubiquitin-activating enzyme (E1) and five ubiquitin-conjugating enzymes (E2s) are indicated. Sizes of protein standards (*M*, in kilodaltons) are shown at right. Minor protein bands immediately below the E1 band are degradation products of E1 (data not shown). *b*, After electrophoretic fractionation of an (E2_{20K})-enriched chromatographic fraction, proteins were eluted either from a gel slice containing the putative E2_{20K} enzyme (lane 2) or from a slice containing the E2_{30K} enzyme (lane 1). The eluted material was incubated in the presence of ATP, ¹²⁵I-labelled ubiquitin, histone H2B and the purified E1 enzyme. Products of the reaction were electrophoresed in an 18% polyacrylamide-SDS gel and detected by autoradiography. Although E2_{30K} can form a thiolester with ubiquitin under the above assay conditions, it fails to conjugate ubiquitin to histone H2B (lane 1 and data not shown). *Ub*, ubiquitin; *uH2B*, monoubiquitinated H2B.

Methods. *S. cerevisiae* strain 20B-12 (ref. 36) was grown in YPD (1% Bacto-yeast extract, 2% Bacto-peptone and 2% glucose) at 30 °C in ~100 l to late exponential phase. Cells were pelleted by centrifugation; the cell pellet (~0.8 kg) was resuspended in 0.5 vol. of 20 mM Na-HEPES (pH 7.2), 0.2 mM 2,4-dinitrophenol (DNP), 20 mM 2-deoxyglucose and incubated for 2 h at 30 °C to deplete intracellular ATP. Cells were washed, resuspended in 1.5 l cold 10 mM Tris-HCl, pH 7.5, and lysed with glass beads in the presence of protease inhibitors⁹. The lysate was clarified by centrifugation at 25,000g for 30 min and stored at -70 °C. DEAE chromatography of the yeast lysate was carried out essentially as for the rabbit reticulocyte lysate²². Proteins eluted with 0.5 M KCl (fraction II) were precipitated by ammonium sulphate, redissolved in 20 ml of 50 mM Tris-HCl (pH 7.5), 5% glycerol, 0.2 mM DTT, 1 mM Na-EDTA, 1 mM ATP, and dialysed overnight at 4 °C against the same buffer (buffer A). Preparation of the ubiquitin affinity column was carried out essentially as described previously²², using purified bovine ubiquitin. Dialysed fraction II was applied to a 4-ml ubiquitin-Sepharose column (~30 mg of ubiquitin per ml) in the presence of 2 mM ATP and 5 mM MgCl₂ in buffer A. After high-salt washes, covalently bound E1 and E2 enzymes were selectively eluted^{22,23} from the column with 50 mM Tris-HCl (pH 7.5), 20 mM DTT. The DTT eluate was concentrated by dialysis against 50 mM Tris-HCl (pH 7.5), 20% polyethylene glycol (20 K, Sigma), 1 mM DTT, and was then further concentrated to 1 ml in buffer A using Centricon 10 (Amicon). This sample (see *a*), containing ubiquitin-activating enzyme (E1, ~3 mg total) and ubiquitin-conjugating enzymes (E2s, 10-30 µg of each) was loaded onto a column (1 × 90 cm) of Sephadex G150 superfine (Pharmacia) equilibrated with buffer A. Aliquots of a fraction (1 µg of total protein) containing E1-dependent ubiquitin-histone conjugating activity were electrophoresed in a 0.5 mm thick, 12.5% polyacrylamide-SDS minigel (Hoefer). To remove SDS, the gel was washed twice for 30 min at ~20 °C in buffer A. The washed gel was cut into slices (~2 mm), and protein-containing slices were identified by Coomassie staining of the edges of the lanes. Proteins were eluted from crushed slices by diffusion into 0.2 ml of buffer A per slice at 4 °C for 20 h. Ubiquitin-histone conjugation in the eluates was carried out in 50 µl of 50 mM Tris-HCl (pH 7.5), 2 mM ATP, 5 mM MgCl₂, 0.2 mM DTT, in the presence of 50 pmol ¹²⁵I-labelled ubiquitin (~1 × 10⁵ c.p.m.), 0.2 µg of bovine histone H2B (Boehringer Mannheim) and 4 pmol of the purified yeast E1 enzyme.



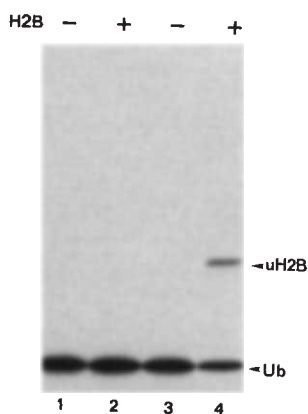


Fig. 3 The *RAD6* gene product is a ubiquitin-conjugating enzyme. *E. coli* cells harbouring pKK223-3 plasmids which either contained or lacked the *RAD6* gene (see Fig. 2 legend) were induced²⁸ and lysed by freeze-thawing in 50 mM Tris-HCl (pH 7.5), 1 mM Na-EDTA, 1 mM DTT. Extracts of cells containing the vector alone (lanes 1 and 2) and the *RAD6*-expressing cells (lanes 3 and 4) were assayed for ubiquitin-histone conjugation as described in the legend to Fig. 1. Products of the reaction were electrophoresed in an 18% polyacrylamide-SDS gel and detected by autoradiography. Designations are as in Fig. 1.

To verify directly that the E2_{20K} ubiquitin-conjugating enzyme is the product of the *RAD6* gene, we cloned this gene using an oligonucleotide probe corresponding to a nucleotide sequence of *RAD6* (ref. 26) outside the observed region of identity between the two proteins (Fig. 2 legend). The cloned gene was verified to be *RAD6* (ref. 27) by restriction mapping (data not shown). A *EcoRI* fragment of ~600 base pairs (bp) containing the entire *RAD6* coding sequence was subcloned downstream of the *tac* promoter in the *Escherichia coli* expression vector pKK223-3 (ref. 28). Extracts from induced cultures of *E. coli* expressing the *RAD6* gene, and control extracts from cells harbouring the same expression vector lacking the *RAD6* insert were assayed for the presence of a ubiquitin-conjugating activity. When supplemented with ATP and purified yeast ubiquitin-activating enzyme, only extracts from *RAD6*-expressing cells mediated the covalent conjugation of ¹²⁵I-labelled ubiquitin to histone H2B (Fig. 3). The remarkable substrate specificity of this ubiquitin-conjugating enzyme is indicated by its apparent failure to ubiquitinate endogenous proteins in the *E. coli* extract (Fig. 3). That there is a distinct ubiquitin-histone conjugating activity in bacterial cells expressing the yeast *RAD6* gene directly identifies *RAD6* as a ubiquitin-conjugating enzyme. This result, together with the close similarity of the molecular sizes and knowledge of the original region of sequence identity between these two proteins, *RAD6* and E2_{20K}, confirms that the E2_{20K} is the product of the *RAD6* gene.

Our findings have identified the *RAD6* gene as a member of a family of closely related genes, which we propose to denote as the *UBC* genes (for ubiquitin-conjugating enzymes). The *UBC* gene family encodes at least five distinct E2 enzymes in yeast (Fig. 1a and unpublished data). The gene encoding the E2_{30K} enzyme (Fig. 1a), cloned previously, was designated *UBC1* (unpublished results). In this terminology, the *RAD6* locus corresponds to the *UBC2* gene which encodes E2_{20K}.

RAD6 function

In *S. cerevisiae*, the *RAD6(UBC2)* gene plays a central role in one of the three epistasis groups of genes involved in DNA repair^{20,21}. Mutations in *rad6* are pleiotropic, and their phenotypic effects include slow growth, severe defects in induced mutagenesis, extreme sensitivity to ultraviolet light, X-rays and chemical mutagens, and hypersensitivity to the antifolate drug trimethoprim^{20,21,29}. In addition, diploids homozy-

gous for some *rad6* alleles are defective in sporulation^{30,31}. That a highly conserved counterpart of E2_{20K} exists in mammals (ref. 5 and our unpublished results) implies that *RAD6*-dependent functions are relevant to all eukaryotic cells. Moreover, our results raise the possibility that some of the human hereditary diseases that involve defects in DNA repair may in fact be due to mutations in genes for specific ubiquitin-conjugating enzymes. For example, in certain types of the disease xeroderma pigmentosum, the cells are apparently defective in an early 'pre-incision' event believed to regulate the initiation of repair processes^{32,33}.

The enzymatic activity of the *RAD6* gene product suggests that at least some of the functions attributed to this gene are mediated by ubiquitination of specific target proteins. Although E2_{20K} ubiquitinates histones such as H2A and H2B *in vitro*, it does not seem to ubiquitinate endogenous proteins in the *E. coli* extract (Fig. 3). The substrate specificity of the *RAD6* protein *in vitro* suggests that its *in vivo* substrates may be few in number, and may include basic proteins such as chromosomal histones. The latter possibility is also consistent with the presence of 14 consecutive negatively charged residues in the carboxy-terminal region of the *RAD6* protein²⁶ (Fig. 2).

In vitro studies with wild-type and mutant mammalian cells defective in DNA repair have emphasized the importance of chromatin structure in regulating the accessibility of lesions in the DNA to the repair enzymes³²⁻³⁵. One possibility is that ubiquitination of proteins mediated by *RAD6(UBC2)* results in the selective degradation of the target proteins. Alternatively, *RAD6(UBC2)*-mediated ubiquitination of chromosomal proteins might induce structural alterations in chromatin directly, thereby allowing access by enzymes of the DNA repair and mutagenesis pathways to the underlying DNA lesions. Targeting of ubiquitination events to the sites of lesions in chromosomes may occur *via* selective exposure of ubiquitination sites in the *RAD6(UBC2)* substrates after localized damage to either DNA or other components of the chromosome. It remains to be determined whether transient ubiquitination events are also required for DNA transcription, recombination, and replication, and, if so, whether specific E2 enzymes are involved.

Sporulation, another distinct pathway defective in certain *rad6* mutants^{30,31}, is accompanied by drastic changes in the protein composition of the forming ascospore, and also by a more compact packing of the chromosomal DNA. This latter process may require changes in the protein composition of the chromatin, possibly analogous to those that occur during spermatogenesis in higher eukaryotes. The requirement for the *RAD6(UBC2)* function in this pathway may thus be due to an involvement of ubiquitination of chromosomal proteins in the process of chromatin remodelling during sporulation.

That the *RAD6* gene product is a ubiquitin-conjugating enzyme underscores the striking functional diversity of the ubiquitin system which is now known to be involved in selective protein turnover, DNA repair, and a variety of other cellular processes, many of which have stress-related functions.

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Note added in proof: The recently cloned yeast cell-division cycle gene *CDC34* encodes a protein whose amino-acid sequence is strongly homologous to that of the *RAD6(UBC2)* protein (B. Byers and M. G. Goebel, personal communication). The *CDC34* protein is also strongly homologous to the ubiquitin-conjugating enzymes other than the *RAD6(UBC2)* protein (data not shown), suggesting that *CDC34* is yet another distinct member of the family of ubiquitin-conjugating enzymes.

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LETTERS TO NATURE

SN1987A supernova: a black-hole precursor?

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The 11+8 neutrino events observed at Kamiokande¹ and IMB² detectors have been analysed by several authors^{1–12}. The inferred total neutrino luminosity W_ν and its distribution among the various neutrino species, vary between the different analyses depending on the events included in the analysis, the parameterization of the neutrino spectra, the role played by ν_e -oxygen scattering, the distance of the Large Magellanic Cloud (LMC) and other aspects. Most authors find high total neutrino energy, in the range $W_\nu = 3\text{--}6.7 \times 10^{53}$ erg, and a large flux of prompt neutronization neutrinos $\Phi_{\nu_e}^{\text{prompt}} \approx 10^{10} \text{ cm}^{-2}$. The Kamiokande collaboration¹ has quoted a value of $W_{\bar{\nu}_e} = 8 \times 10^{52}$ erg for the total $\bar{\nu}_e$ energy. This value is subject to uncertainties in the distance to the LMC, low statistics and other experimental and theoretical factors. From this value we have deduced⁸ that $W_\nu \approx 6.7 \times 10^{53}$ erg which implies a gravitational energy release $\geq 0.37 M_\odot c^2$. This value is too high for a binding energy of a standard, $1.4 M_\odot$, neutron star which means that a massive $M \geq 2 M_\odot$ neutron star or a black hole form. In the following we focus on lepton number conservation and the ensuing limitation on $\Phi_{\nu_e}^{\text{prompt}}$. We show that an identification of the first two events in the Kamiokande data as ν_e prompt events requires a mass, $M \geq 6 M_\odot$, which implies a black-hole formation. This is also consistent with the probable identification of the progenitor¹³ as a B3I supergiant of $\sim 25 M_\odot$.

Several authors (refs 3, 10; A. Dar and S. Dado, personal communication) attribute the first two forward-directed Kamiokande events ($\theta_1 = 18 \pm 18^\circ$, $\theta_2 = 15 \pm 27^\circ$) to ν_e -e scattering. The probability that these events originate from $\bar{\nu}_e$ -p reaction, or from ν -oxygen scattering with an isotropic distribution is, even using the higher limit for the angles, $P = p(\theta_1 \leq 36^\circ)p(\theta_2 \leq 42^\circ) = 0.012$. As these two neutrino events come within the first 0.1 s it is suggestive to identify them as prompt neutronization neutrinos generated via $e^- + p \rightarrow \nu_e + n$ during the collapse. (Thermalized neutrinos contain equal numbers of $\bar{\nu}_e$ and ν_e and interpreting the two events as thermal neutrinos scattering on electrons would require—due to the

much larger $\bar{\nu}_e + p \rightarrow e^+ + n$ cross-section, about 50 isotropic events within the first 0.3 seconds³). The resulting energy in neutronization neutrinos is then $W_{\nu_e}^{\text{prompt}} \approx (1.5 \pm 0.3) \times 10^{53}$ erg with corresponding fluxes $\Phi_{\nu_e}^{\text{prompt}} \approx 1.2 \pm 0.2 \times 10^{10} \nu_e \text{ cm}^{-2}$ (with average energies $E_{\nu_e}^{\text{prompt}} \approx 25$ MeV) are much higher than the predictions of the conventional model calculations involving a neutron star formation^{3,10}. Thus if we insist on this natural identification of the first two events, a neutron star formation is inconsistent.

We would like to reinforce this argument in a model-independent way. The prompt neutrino flux is limited (due to lepton number conservation) by the number of electrons in the collapsing core. Numerical simulations¹¹ show that $\sim 0.6 \nu_e$ prompt per electron are obtained, but even when assuming a 1:1 ratio one still has $\Phi_{\nu_e}^{\text{prompt}} \leq (M_{\text{core}}/M_\odot) (L/50 \text{ kpc})^{-2} (0.2 \times 10^{10}) \nu_e \text{ cm}^{-2}$. Thus $\Phi_{\nu_e}^{\text{prompt}} \approx 1.2 \times 10^{10} \text{ cm}^{-2}$ requires $M_{\text{core}} \geq 6 M_\odot$ implying a black hole. This argument relies on two events and is therefore of limited statistical significance. For a canonical neutron-star mass of $1.4 M_\odot$, one expects 0.25 prompt neutrinos and the Poisson probability of seeing two neutrinos $P = [(0.25)^2 e^{-0.25}]/2 = 0.024$. A similar probability of 0.025 is obtained for a standard neutron star taking the first event to be a prompt ν_e and the second a $\bar{\nu}_e$ event. Even though these probabilities are small and may indicate a black-hole formation, they are not small enough to exclude completely a standard neutron star.

Here one should note that supporting evidence for a possible black-hole model is also supplied from arguments other than the lepton number conservation. The first is the already mentioned apparent high total neutrino energy⁸. The second is the probable identification of the SN1987A progenitor as Sk-69 202¹³, a blue supergiant of spectral type B3I^{14,15} implying¹⁶ a mass $\sim 25 M_\odot$. This identification is consistent with the observed lower than standard optical luminosity, the higher than standard expansion velocity and the light-curve time behaviour^{17–19}. Simulations by Wilson *et al.*²⁰ indicate that progenitors with masses $\geq 25 M_\odot$ produce a massive iron core which upon cooling through neutrino emission followed by accretion, form a black hole on a time scale of a few seconds. In this connection it is interesting to note that a $25 M_\odot$ progenitor²⁰ implies $W_{\nu_e} = 11 \times 10^{52}$ erg (ref. 21) leading to $W_\nu = 9 \times 10^{53}$ erg. Therefore, such a progenitor is consistent with our seemingly high previous estimate⁸ of W_ν . The third argument concerns the 7-s gap between the first eight and the last three events of the Kamiokande data. This may be due to a cooling of a neutrino sphere^{11,12}, however it has recently been argued²² that it is unlikely that the last three events are the tail of a cooling process, thus suggesting other causes for this gap. For example, a late

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accretion phase, or a rotating collapse leading to two neutron stars which eventually coalesce^{23,24}. In these cases a black hole is a probable outcome.

Even though a black-hole outcome seems to be favoured we cannot rule out a standard neutron-star formation. If indeed a neutron star will eventually be inferred from X-ray observations (within the next couple of years), very important hints concerning novel particle physics features of neutrinos can be deduced⁸. We need to minimize effects which diminish W_ν^{total} and/or Φ_ν^{prompt} . A ν_e magnetic moment $>10^{-14} \mu_e$ (10^{12} gauss/B) where B is the magnetic field near the core reduces, by mixing sterile right-handed neutrinos, both W_ν and Φ_ν^{prompt} by a factor of two. MSW^{25,26} or vacuum ν -mixing can enhance the detectability of the thermal component^{4,5} but reduce Φ_ν^{prompt} by mixing ν_μ or ν_τ into the initial pure ν_e neutronization neutrinos. A feature which ameliorates the W_ν problem by enhancing the total $\bar{\nu}_e$ signal and also enhance somewhat Φ_ν^{prompt} is ν_τ , ν_μ decays, that is $\nu_\tau \rightarrow \nu_e + M^0$, $\nu_\mu \rightarrow \nu_e + M^0$ with M^0 an unobserved majoron⁸.

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Photon-photon pair production and the opacity of SN1987A to TeV and PeV γ -rays

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Supernovae have long been considered as likely sites of cosmic-ray acceleration. Interaction of newly accelerated cosmic-ray nuclei with target material within the expanding supernova is expected to produce an observable flux from SN1987A of very high-energy and possibly ultra high-energy γ -rays in the TeV and PeV (10^{15} eV) ranges, and several experiments are being constructed to detect this radiation. The presence of intense infrared emission from the supernova itself will, however, make some regions of SN1987A opaque to TeV and PeV γ -rays due to pair-production interactions. Observations at these energies, combined with a knowledge of which regions of SN1987A could be contributing γ -rays may thus give information about the nature and location of particle accelerators in supernovae. Here I discuss the important question of photon-photon pair-production interactions and calculate from which regions of SN1987A may be observed TeV and PeV γ -rays.

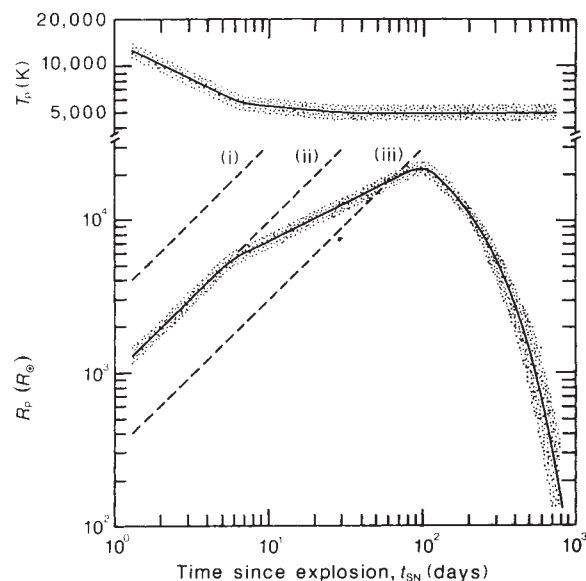


Fig. 1 Approximate photospheric radius and temperature of SN1987A based on observations and expected behaviour (see text). Dashed lines indicate shells expanding ballistically at (i) 25,000 km s⁻¹ (fastest material detected), (ii) 8,000 km s⁻¹ (shell containing most of the matter), and (iii) 2,400 km s⁻¹ (probable boundary between H envelope and He layer).

Because of the relatively close location of SN1987A in the Large Magellanic Cloud¹ (LMC), if only a small fraction of the $\sim 10^{51}$ erg released during a typical type II supernova explosion^{2,3} were converted to very energetic particles then SN1987A could be observable at TeV energies with existing southern hemisphere detectors. Several possible mechanisms for acceleration in supernovae are discussed in ref. 4. If $\sim 10^{39}$ erg s⁻¹ was used to accelerate protons with a spectrum extending up to 10 TeV then the supernova could be observable even now above 1 TeV (ref. 5). If the particle spectrum extends beyond 10 PeV then existing air shower experiments might also detect the supernova and data from the Buckland Park air shower array⁶ at Adelaide have been examined for a burst of PeV γ -rays arriving within a short time interval of the observed neutrino burst^{7,8}. This search is continuing for delayed PeV emission from SN1987A.

From mid-latitudes SN1987A is viewed at large zenith angles and this increases the effective energy threshold of a typical air shower array to >1 PeV. Interaction of γ -rays with photons of the cosmic microwave background radiation, however, severely reduce the flux of γ -rays from LMC at energies $\geq 10^{14}$ eV (see ref. 9 and refs therein). Air shower observations from particle arrays at much more southerly latitudes and higher altitudes, such as from the particle array under construction at the South Pole^{10,11}, making observations possible at 10^{14} eV, or below, are therefore highly desirable. To observe ultra high-energy γ -rays from SN1987A, new particle detectors are being installed at Mount Chacaltaya, Bolivia, and by a Japan-New Zealand-Australia collaboration in New Zealand, but it is doubtful whether their energy thresholds for air showers from SN1987A will be much <1 PeV.

Observations were carried out by the Adelaide group in April 1987 using the White Cliffs Solar Power Station¹², Australia, as a γ -ray telescope operating at ~ 5 TeV and these will resume in September; attempts are being made to speed up development of a new telescope to be located at Woomera¹³, Australia. Other detectors which may be used to search for γ -ray emission from SN1987A are the University of Durham telescope¹⁴ at Narrabri, Australia, and the telescope of Potchefstroom University¹⁵, South Africa, both of which operate in the TeV energy range.

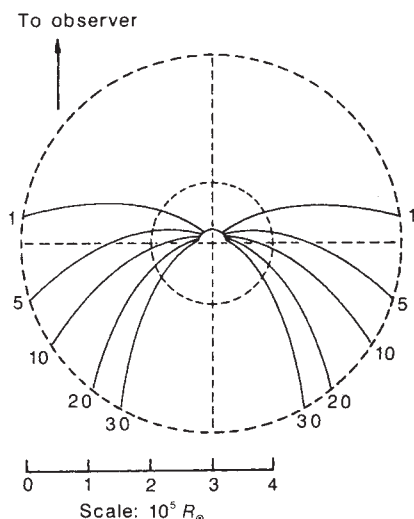


Fig. 2 Contour map showing optical depth of 1 TeV γ -rays to photon-photon collisions throughout the supernova at a time of 100 days after the supernova explosion. Numbers attached to contours give the optical depth. The inner and outer dashed circles represent the location of shells expanding at 8,000 and 25,000 km s^{-1} respectively. The observer is located in the direction towards the top of the page.

It is therefore timely to consider the opacity of various regions of SN1987A to TeV and PeV γ -rays.

Apart from the presence of absorption and emission lines, the spectrum of SN1987A has been consistent with that emitted by a black body. Presently (~ 100 days after explosion) the best fitting temperature is $\sim 5,000$ K (M. A. Dopita, personal communication). The absorption of γ -rays in black-body radiation at this temperature peaks at ~ 1 TeV, in the important energy range of atmospheric Cerenkov telescopes. To calculate the transmission probability of a γ -ray one needs to know the intensity of radiation as a function of frequency, I_ν , in all directions at all points along the γ -ray's trajectory. I assume that within the photosphere the intensity in all directions is equal to that of black-body radiation, $B_\nu(T_p)$, at the photospheric temperature, T_p . Outside the photosphere the intensity may be approximated by $B_\nu(T_p)$ in directions towards the photosphere and zero in all other directions.

The photospheric temperature and radius may be obtained from infrared and optical observations of the supernova by assuming that the luminosity is given by $L = 4\pi R_p^2 \sigma T_p^4$ where σ is the Stefan-Boltzmann constant, and R_p is the radius of the photosphere. T_p and R_p are available for the period ~ 6 –16 days after the explosion from infrared photometry¹⁶ carried out at the European Southern Observatory. Before ~ 6.5 days after the explosion the luminosity was approximately constant with the photosphere expanding uniformly¹⁷ at $\sim 8,000$ km s^{-1} . The luminosity was $\sim 8 \times 10^{41}$ erg s^{-1} at 70 days¹⁷ and $\sim 1.1 \times 10^{42}$ erg s^{-1} at 100 days (M. A. Dopita, personal communication) after the explosion. Since ~ 30 days after the explosion the photospheric temperature has remained constant at $\sim 5,000$ K characteristic of the temperature of recombination of hydrogen and is not expected to depart dramatically from this value as the recombination front passes through the helium and inner layers of the core. At present the supernova appears to be fuelled by the radioactive decay of ^{56}Co due to decay of ^{56}Ni produced by explosive nucleosynthesis and its luminosity is expected to decline exponentially with a decay time of ~ 60 –84 days, more typical of a type I supernova.

Approximations to the photospheric temperature and radius based on these observations and the expected behaviour are plotted against time since the explosion in Fig. 1. At least during the first few years the remnant may be considered to expand

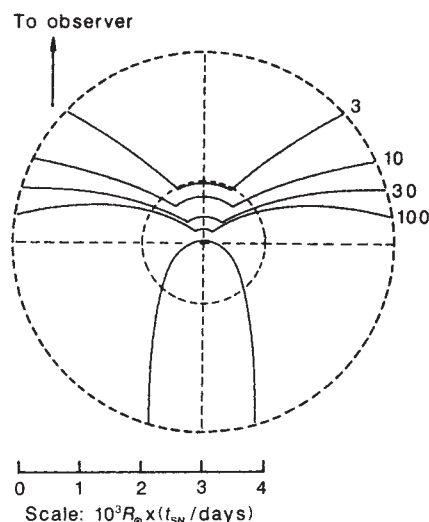


Fig. 3 Evolution of the region of the supernova which is transparent to 1-TeV γ -rays. The curves show the boundary of regions with optical depth less than one. The numbers attached to the curves are the days after supernova explosion.

ballistically and so lines of constant expansion velocity will correspond to the boundaries between different mass shells. Also plotted in Fig. 1 are lines of constant expansion velocity at 8,000 and 25,000 km s^{-1} , the former being likely to contain essentially all of the hydrogen envelope, the latter corresponding to the fastest material observed during the initial expansion phases¹⁷. At ~ 70 days after the explosion the photosphere appeared to have entered the He layer¹⁷. Based on the photospheric radius at ~ 70 days, the boundary between the H envelope and the He layer corresponds to an expansion velocity of $\sim 2,400$ km s^{-1} and this is also indicated in Fig. 1.

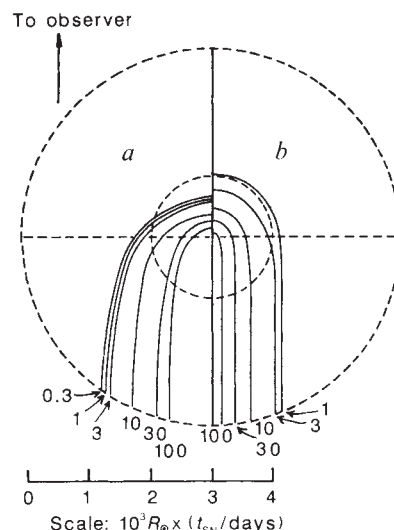
The problem of the transparency of a supernova to photon-photon collisions was discussed briefly in ref. 18. Here a detailed calculation relevant to SN1987A is given. The optical depth of γ -rays of energy E_γ to photon-photon collisions at a particular location within the supernova is given by

$$\tau_{\gamma\gamma}(E_\gamma) = \frac{1}{hc} \int \int_{\Omega_p} \int \frac{B_\nu(T_p)}{\nu} \sigma_{\gamma\gamma}(E_\gamma, \nu, \theta) (1 - \cos \theta) d\nu d\Omega ds$$

where the integral over s is along the path of the γ -ray from the location in question to the observer, Ω_p is the solid angle subtended by the photosphere at the γ -ray, $\sigma_{\gamma\gamma}$ is the pair-production cross-section¹⁹, and θ is the angle between the directions of the γ -ray and the low-energy photon. Using the model of photospheric temperature and radius given by the solid lines in Fig. 1 the optical depth of γ -rays of energy 1 TeV is contoured in Fig. 2 at various points within the expanding supernova 100 days after the explosion. Note that the $(1 - \cos \theta)$ term allows one to see TeV γ -rays produced close to the part of the photosphere nearest the observer whereas a large region behind the photosphere is inaccessible to observation.

The time evolution of the region of the supernova visible from Earth is shown in Fig. 3. Note that at TeV energies the photosphere is essentially opaque to γ -rays. As the photosphere expands less rapidly than the supernova, and at later times will actually contract, a successively larger fraction of the supernova becomes accessible to observation. Of course, γ -rays or nuclei produced deeper into the supernova might still produce an observable signal as a result of electron-photon cascading (being fed by hadronic interactions in the case of a primary proton or nucleus) involving pair production on photons or nuclei and bremsstrahlung or Compton scattering. This possibility should

Fig. 4 *a*, Evolution of the region of the supernova which would be transparent to 1-PeV γ -rays in the absence of matter (see Fig. 3 for explanation). *b*, Evolution of the region of the supernova which is less than approximately one radiation length from the observer. The numbers attached to the curves are the days after the supernova explosion, t_{SN} . Note that the assumption of ballistic expansion also allows this figure to be used to obtain the number of radiation lengths, $\tau_{\gamma\text{N}}$, to points within the supernova at given times after explosion by regarding the numbers attached to the curves as $\tau_{\gamma\text{N}}^{0.5} t_{\text{SN}}$.



be borne in mind although, because of the higher-energy primary particles required for a given observed γ -ray energy, I will not consider this process further here.

Whether, and when, we would expect to see γ -rays will depend on where the acceleration takes place, the time evolution of the luminosity in high-energy particles, and the efficiency with which high-energy charged particles are confined in the supernova shell by magnetic fields. To produce γ -rays efficiently, the cosmic rays must traverse at least about one interaction length before escaping from the supernova or losing a significant fraction of their energy by adiabatic expansion losses. Target material is readily available in the hydrogen envelope which is largely contained within the shell expanding at $\sim 8,000 \text{ km s}^{-1}$, and TeV γ -rays produced within this region would only have become observable three days after the explosion. If acceleration of particles in the very outer layers of the supernova took place during the explosion by the mechanism²⁰ suggested for type I supernovae, TeV γ -rays could have been observable early on. Although SN1987A is type II, this possibility should perhaps not be completely ruled out, because of the unusual nature of the pre-supernova star, a blue supergiant¹, whose stellar wind may have cleared a surrounding cavity. Similarly, TeV γ -rays could have been seen from interactions of particles accelerated by second-order Fermi acceleration in the outer regions of the expanding supernova²¹, or by first-order Fermi acceleration at the shock²² (see ref. 23 for a review). In all cases, the TeV γ -ray flux expected would, however, be reduced significantly by photon-photon collisions.

Results for 1-PeV γ -rays are plotted in Fig. 4a. At this higher energy the absorption length in black-body radiation at a temperature typical of the photosphere is considerably longer. This means that it is, in principle, possible to observe PeV γ -rays produced part of the way into the photosphere. How far into the photosphere one can observe in practice will depend on the matter-density profile and composition which determine the optical depth to photon-nucleus pair production. This density profile is rather uncertain but some information is available from the time dependence of the photospheric radius. From a preliminary analysis (M. A. Dopita, personal communication) the density appears to drop off with radius, R , as R^{-5} in the $\sim 0.2 M_{\odot}$ hydrogen envelope and as R^{-3} in the helium layer. Based on the almost ballistic expansion of the photosphere at early times, the density beyond $\sim 8,000 \text{ km s}^{-1}$ must drop off much more rapidly, the preliminary analysis indicating $\sim R^{-15}$. Using this density profile to represent the entire matter distribution in the supernova one can estimate the number of radiation lengths between the observer and points within the supernova. Neglecting a separate treatment of layers interior to the He layer will not affect the estimates except for regions inside the shell

moving at $\sim 1,000 \text{ km s}^{-1}$ and will not affect the conclusions drawn here.

The result is given in Fig. 4b which clearly indicates that at times $\lesssim 30$ days after the explosion the opacity due to photon-nucleus pair production cannot be neglected. In particular, for a burst of PeV γ -rays to have been observed during the first day after the explosion, PeV γ -rays must have been produced outside the $\sim 8,000 \text{ km s}^{-1}$ shell.

Finally, if a pulsar is present and is accelerating high-energy particles^{24,25}, γ -rays could be expected when the particles have had time to diffuse out to the region which is optically thin at the energy in question. Of course, if the inner part of the shell is too dense then the energetic particles will lose their energy by pion production, for example, in a region from which any γ -rays produced might not escape. But if γ -rays are detected one could place limits on the diffusion coefficient by assuming particles were accelerated by a pulsar which turned on soon after the explosion, and by knowing the radius at which the supernova becomes transparent to γ -rays.

Whatever the acceleration mechanism, in estimating the production rate of high energy particles from an observed flux of γ -rays it will be necessary to correct the observed flux for photon-photon collisions both with the cosmic microwave background radiation on traversing the distance from the LMC to the Earth, and with the infrared radiation from the supernova itself. It is unfortunate that nature has conspired to make the absorption greatest by the first mechanism at 1 PeV and by the second at 1 TeV, the energies at which ground-based observations using air shower arrays and atmospheric Cerenkov telescopes are most effective.

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Cloud optical depth feedbacks and climate modelling

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Recent general circulation model studies^{1–3} performed to assess the equilibrium climate response to doubling atmospheric CO₂ suggested a global mean surface warming of 3.5–4.2 °C. Part of this warming was attributed to a change in cloud cover¹. But all of these studies neglected changes of cloud optical properties which were shown to provide a substantial negative feedback in radiative-convective models if the cloud liquid water content was assumed to increase with increasing temperature^{4,5}. This hypothesis is examined in a climate model where clouds are simulated interactively with dynamics, radiation and hydrological cycle⁶. The thermal forcing is introduced by a 2% increase of the solar constant which is equivalent to a doubling of CO₂¹. The results show the anticipated increase of cloud liquid water and cloud optical depth. A feedback analysis of the simulated climate change supports earlier suggestions of the importance of cloud optical depth feedbacks^{4,5}. The net effect of clouds is to provide a negative feedback on surface temperature, rather than the positive feedback found in earlier general circulation model studies without considering cloud optical depth feedbacks¹.

The characteristics of the climate model used in this study are similar to those quoted above^{1–3}. It consists of (1) a general circulation model of the atmosphere, (2) a mixed-layer ocean model and (3) a sea-ice model. The model has a global computational domain with realistic topography. The governing equations for the atmosphere are solved by finite-difference techniques at three levels in the vertical and on a spherical grid of 11.25° resolution. Turbulent surface fluxes of heat, water vapour and momentum are calculated from Ekman layer similarity theory. Convective energy fluxes developing in conditionally unstable air are related to the water vapour supply in the planetary boundary layer. Solar and terrestrial radiation fluxes

are calculated at eight levels using the two-stream formulation of the flux equations⁷. The distributions of ozone, carbon dioxide and aerosols are prescribed according to climatology while those of water vapour and clouds are computed from the respective budget equations. The diurnal and seasonal cycles of incoming solar radiation are included. Land surface properties are either predicted (temperature, wetness, snow amount) or prescribed (albedo).

A novel feature in climate modelling is the cloud scheme applied. It is based on the numerical solution of the cloud liquid-water continuity equation including simple parameterizations for partial cloud cover and cloud microphysical terms such as condensation of water vapour, evaporation of cloud droplets and rain drops and conversion of small droplets to large rain drops by coalescence^{6,8}. No distinction is made between liquid and ice phase except that we take into account an efficient diffusional growth of ice crystals in the temperature range of ~0 °C to –20 °C where a mixed phase of cloud droplets and ice crystals is supposed to occur⁹. Cloud radiative properties such as shortwave albedo and longwave emissivity are parameterized according to Stephens¹⁰ in terms of the cloud liquid-water content which forms a reliable basis for such a parameterization in most parametric ranges. Thus, other than in most general circulation models, the cloud radiative properties are linked to the rest of the model physics via cloud dynamics.

The sea surface temperatures are computed from the heat balance of the ocean mixed layer which is allowed to vary in depth according to the local energy budget and momentum input at the surface¹¹. The ocean heat transport is prescribed as constant in time at every grid point according to a value obtained from a simulation with seasonally varying climatological sea surface temperatures¹. This procedure implies vanishing heat divergence in a steady state which is close to the observed, thus permitting a realistic simulation of the long-term mean sea surface temperature distribution. But, because we apply the method also in a climate perturbation experiment, ocean transport feedbacks cannot be considered. The lateral and vertical growth or decay of sea ice is computed from the local energy budget, separately for open water and ice in each grid box.

To assess the feedbacks resulting from radiative forcing we conducted two experiments with different values of the solar constant, a 20-yr control simulation with the present value of $S_0 = 1,370 \text{ W m}^{-2}$ and a 30-yr perturbation experiment with the increased value of $S_0 + 2\% = 1,397.4 \text{ W m}^{-2}$. The results will be presented as averages over the last 10 years of each run.

Table 1 shows a set of observed and simulated climate variables relevant for understanding the feedback mechanisms resulting from the forcing. Obviously, the control simulation successfully reproduces the observations. All parameters except the planetary albedo with a too low value are simulated within observational uncertainties. This is desirable because the induced climate change is likely to depend on the global mean control climate¹². The 2% increase of the solar constant causes

Table 1 Observed and simulated globally averaged climate parameters

	Surface temperature (°C)	Precipitation (mm per day)	Total cloud cover (%)	Total liquid-water path (g m ⁻²)	Planetary albedo (%)	Cirrus cloud emissivity (%)	Arctic sea ice extent (10 ⁶ km ²)	Antarctic sea ice extent (10 ⁶ km ²)
Observed	14.9	2.7	~50	—	30.0	—	11.3	11.8
Control experiment	14.7	2.5	53.4*	43.2	27.8	42.4	11.9	12.0
Perturbation – control	3.32	0.15	0.50*	4.52	0.31	17.4	–3.06	–6.04
250 hPa	5.53	—	1.56	1.17	—	—	—	—
550 hPa	4.30	—	–0.78	1.67	—	—	—	—
850 hPa	3.26	—	–1.00	1.68	—	—	—	—

The last three rows show in addition the vertical distribution of the difference (perturbation – control) of temperature, cloud cover, and cloud liquid-water path. Surface temperature data from ref. 16, precipitation data from ref. 17, albedo from ref. 18, Arctic ice extent from ref. 19 and Antarctic ice extent from ref. 20.

* Random overlap.

Table 2 Change of net radiation budget $\delta_x R$ due to changes of individual parameters x in the perturbation experiment

	x = solar constant	x = temperature	x = water vapour	x = clouds	x = surface albedo	$\sum_i \delta_{x_i}$	$\delta \sum_i x_i$
Planet							
$\delta_x R$	4.6	-12.4	4.5	4.5	0.6	1.7	2.1
$\delta_x S / -\delta_x F$	(4.6/0)	(0/-12.4)	(0.3/4.2)	(-2.2/6.7)	(0.6/0)	(3.3/-1.6)	(2.8/-0.7)
Atmosphere							
$\delta_x R$	1.5	-9.4	-0.4	6.5	-0.4	-2.2	-1.8
$\delta_x S / -\delta_x F$	(1.5/0)	(0.3/-9.7)	(0.7/-1.1)	(-0.3/6.8)	(-0.4/0)	(1.8/-4.0)	(1.7/-3.5)
Surface							
$\delta_x R$	3.1	-3.0	4.9	-2.0	1.0	4.0	3.9
$\delta_x S / -\delta_x F$	(3.1/0)	(-0.3/-2.7)	(-0.4/5.3)	(-1.9/-0.1)	(1.0/0)	(1.5/2.5)	(1.1/2.8)

The respective solar ($\delta_x S$) and terrestrial ($-\delta_x F$) radiation changes are given in brackets. The last two columns compare the sum over the individual changes and the change resulting from changing the parameters all at once. Positive (negative) values indicate warming (cooling). The values are obtained by global and time averaging the respective changes over the diurnal and annual cycles. Units are Wm^{-2} .

a global warming of 3.3°C at the surface increasing with height. Evaporation and hence also precipitation increases due to the increase of the surface saturation mixing ratio with temperature according to the Clausius-Clapeyron relation. The increase in surface temperature causes part of the sea ice to melt, approximately 25% in the Arctic and 50% in the Antarctic regions. Total cloud cover is slightly larger due to an overall increase of high clouds and a high-latitude increase of low clouds. In the remaining areas cloudiness decreases. The total liquid-water path is larger by about 10% causing an increase of planetary albedo and cirrus cloud emissivity. The change in emissivity of middle and low clouds (not shown) is negligible because in the control run they are nearly black.

The most interesting aspect of the perturbation run is the increase of cloud liquid water with increasing temperature which is assumed in various radiative-convective model studies^{4,5}. From aircraft soundings of cloud liquid water content L and temperature T made in the Soviet Union¹³, Somerville and Remer⁵ derived a value $f \equiv 1/L \, dL/dT \approx 0.04 \, \text{K}^{-1}$. Our model experiments suggest an increase of globally averaged f with height of (0.03/0.06/0.10) K^{-1} for (low/middle/high) clouds. Whether the rather large f for high clouds is realistic cannot be decided from the soundings mentioned above which end already at an altitude of 6–7 km. The main conclusion of Somerville and Remer⁵ is that the temperature dependence of cloud liquid-water content causes a negative feedback which could reduce the CO_2 -induced warming by as much as 50%. This result is obtained with a model which does not allow for changes of cloud cover and cloud emissivity. In our experiments both parameters vary as does cloud albedo so that the sign of the net cloud feedback may be negative or positive.

To evaluate the cloud feedbacks the equilibrium climate states evolving from the control and perturbation experiments are analysed in terms of the global radiation budget¹⁴. This procedure involves two idealizations. First, the time mean radiative flux is represented by the flux computed from the equilibrium climate of the control and perturbation run, respectively.

Second, the net solar and terrestrial flux differences between perturbation and control, δS and δF , are approximated by a sum of partial differentials each of which represents a flux difference, $\delta_x S$ and $\delta_x F$, due to a change of a relevant climate parameter x , such as solar constant, temperature, water vapour mixing ratio, cloud parameters and surface albedo. The globally averaged results are shown in Table 2, separately for the planet, the atmosphere and for the Earth's surface. The change of clouds induces a substantial greenhouse warming in the atmosphere but a cooling of the surface caused by a reduced solar radiation input at the surface. The resulting cloud feedback for the planet is positive, just as large as the water vapour feedback and the direct solar forcing. Summation over the individual changes approximately equals the total change (last column) computed from the perturbation experiment. Unfortunately, the net planetary radiation budget is not zero. The residual is probably caused by inconsistencies due to interpolations of, for example, temperature, fluxes between the dynamic part and the radiation part of the model. At the surface the residuals are balanced by increased turbulent fluxes of sensible and latent heat.

For a closer inspection of the cloud feedbacks we subdivide the total cloud change into four parts representing the changes of cloud cover and cloud liquid water separately for high clouds and middle/low clouds (Table 3). Again, summation over the individual changes gives approximately the correct net change (last column) so that the splitting seems to be justified. The liquid-water increase of high clouds and the resulting increased infrared emissivity (Table 1) produce a substantial greenhouse warming of the atmosphere. At the surface, however, the increased albedo of all types of clouds causes a net cooling, as anticipated^{4,5}, which overcompensates the heating induced by the low-latitude reduction of low and middle cloud cover. Thus, the sign and the magnitude of the net cloud feedback are essentially governed by the cloud optical depth feedback.

The redistribution of cloud amount and the increase of cloud liquid-water content in the perturbation climate are in correspondence with previous conjectures¹⁻⁵; however, the

Table 3 Change of net global radiation budget $\delta_x R$ due to changes of individual cloud parameters x in the perturbation experiment

	x = high cloud cover	x = middle and low cloud cover	x = high cloud liquid water	x = middle and low cloud liquid water	$\sum_i \delta_{x_i}$	$\delta \sum_i x_i$
Planet						
$\delta_x R$	0.5	1.3	3.6	-1.2	4.2	4.5
$\delta_x S / -\delta_x F$	(-0.4/0.9)	(1.9/-0.6)	(-2.3/5.9)	(-1.2/0)	(-2.0/6.2)	(-2.2/6.7)
Atmosphere						
$\delta_x R$	0.9	-0.3	5.1	0.2	5.9	6.5
$\delta_x S / -\delta_x F$	(0.1/0.8)	(-0.7/0.4)	(-0.2/5.3)	(0.1/0.1)	(-0.7/6.6)	(-0.3/6.8)
Surface						
$\delta_x R$	-0.4	1.6	-1.5	-1.4	-1.7	-2.0
$\delta_x S / -\delta_x F$	(-0.5/0.1)	(2.6/-1.0)	(-2.1/0.6)	(-1.3/-0.1)	(-1.3/-1.4)	(-1.9/-0.1)

The respective changes of solar ($\delta_x S$) and terrestrial ($-\delta_x F$) radiation are given in brackets. Units are Wm^{-2} .

observational basis for validating these results is very poor. Moreover, although the cloud feedbacks analysed above seem plausible the question remains: are they realistic—will the real atmosphere respond to a radiative perturbation (induced by a CO_2 increase, for instance) in a similar way? We cannot answer this question but there is evidence that the cloud radiative forcing (the effect of clouds on the radiation budget) is realistic in the control climate. Our estimates based on the computation of the planetary radiation budget with and without clouds are in broad agreement with many estimates based on satellite observations¹⁵. In the global and annual mean the net cloud effect is a cooling of the planet because the albedo effect (54 W m^{-2}) dominates over the greenhouse effect (31 W m^{-2}) by 23 W m^{-2} .

Nevertheless, the results should be taken as preliminary because we used a coarse-resolution model with many simplifications, particularly in the cloud microphysical part. Much more theoretical and observational work seems to be necessary to develop and validate cloud prediction algorithms which are based on the governing physical principles.

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Southern oscillation simulated in a global climate model

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The Southern Oscillation is the dominant pattern of interannual climatic variation over the Earth. Climatic variance associated with this phenomenon is exceeded only by the annual cycle and the ice ages. Although there is general agreement among climatologists that the Southern Oscillation originates in the interaction between the atmosphere and the tropical ocean, the detailed mechanism(s) have remained elusive. In this report we present evidence that the essential aspects of the Southern Oscillation are simulated in a general circulation model (GCM) of the coupled atmosphere and upper ocean. This indicates that: (1) the physical basis of this global oscillation is present in the equations of motion underlying this model; (2) multi-year simulations of climate by GCMs, which have hitherto primarily been used to study equilibrium properties of climate, may also be useful in investigating time-dependent changes on the interannual timescale.

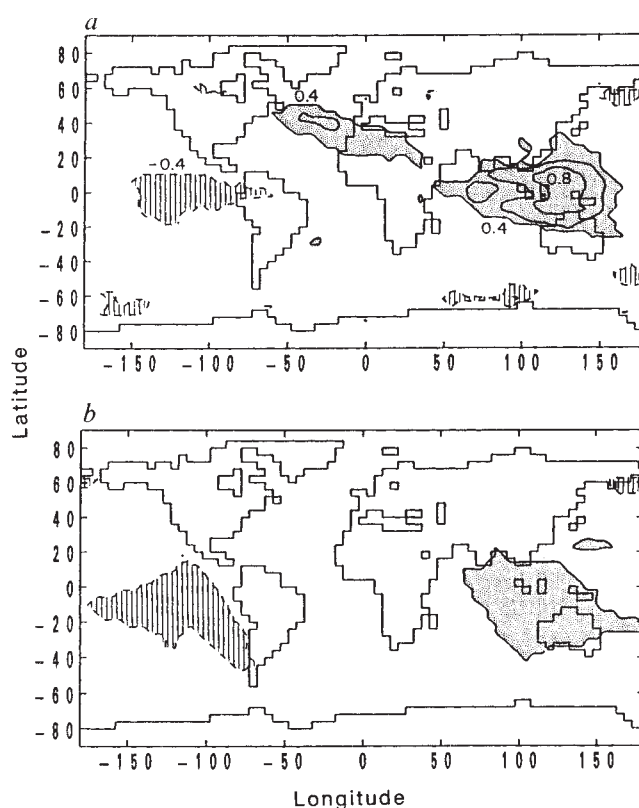


Fig. 1 *a*, Correlations of annual average sea-level pressure (SLP) with that at 130°E , 2°S from a 23-year simulation of the OSU coupled atmosphere-upper ocean GCM. Only those correlations significant at $\geq 95\%$ confidence limit are shown. The contour spacing is 0.2. The spatially coherent anticorrelation between the Indonesia-Australia region and the eastern Pacific Ocean is a manifestation of the Southern Oscillation that is also found in the observed pressure field. *b*, Observed sea-level pressure isocorrelations with Darwin, Australia, after Wright²⁰.

It has been suggested that quasi-periodic variations of the trans-Pacific pressure gradient result in weakening (strengthening) of the trades causing easterly (westerly) migration of the zone of convergence and precipitation¹. Wyrki^{2–6} hypothesized that the variations of wind stress on the ocean give rise to quasi-oscillatory behaviour of east-west water transport and temperature gradient in the eastern Pacific, which in turn redistributes large amounts of energy affecting global weather. Several models of the Southern Oscillation have been developed by simulating atmosphere-ocean interaction in the equatorial Pacific basin^{7–12}. Cane and Zebiak¹¹ and Cane *et al.*¹² have reported a coupled atmosphere-ocean model of the equatorial Pacific region which reproduces major features of the Southern Oscillation, including interannual variations of sea surface temperature (SST). In their model, mean monthly conditions are specified from climatological data and deviations from the mean are calculated according to physical laws. Also, several authors have reproduced atmospheric characteristics of the Southern Oscillation in GCMs by prescribing anomalously high SSTs in the model eastern Pacific^{13–17}. The results we report here are different in that a global atmosphere-ocean circulation model was integrated without any empirical constraints on the simulated interannual variations.

Walker and Bliss¹⁸ characterized the Southern Oscillation as '... when pressure is high in the Pacific Ocean, it tends to be low in the Indian Ocean from Africa to Australia ...'. This large-scale swaying of pressure between the two oceans is the primary atmospheric signature of the Southern Oscillation. It results in sea-level air pressure near Indonesia and Australia to

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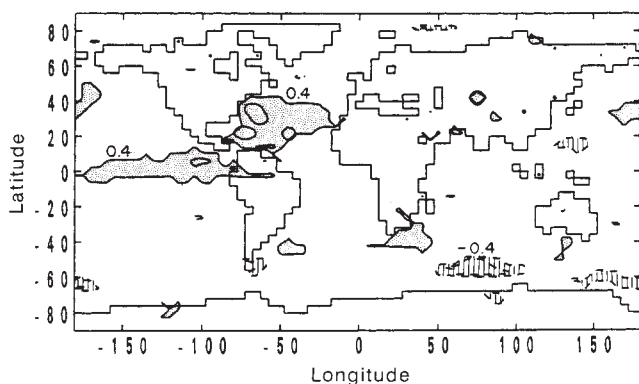


Fig. 2 Correlations of annual average surface-level temperature with annual average sea-level pressure at 130° E, 2° S. Only correlations significant at $\geq 95\%$ confidence level are shown. The contour spacing is 0.2.

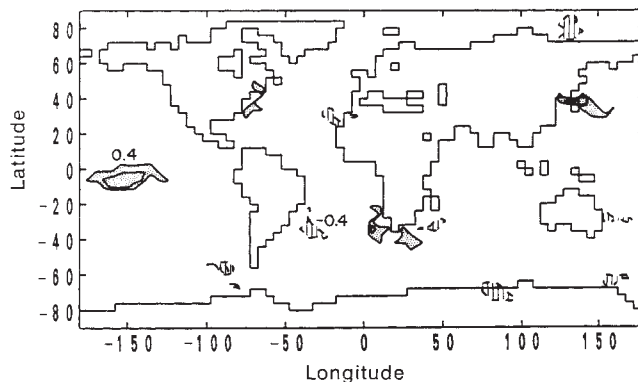


Fig. 3 Correlations of annual average precipitation with annual average sea-level pressure at 130° E, 2° S. Only correlations significant at $\geq 95\%$ confidence level are shown. The contour spacing is 0.2.

be anti-correlated with air pressure over a large region of the eastern equatorial Pacific, off the west coast of South America (see Fig. 8.1:4 of Hastenrath¹⁹ and Fig. 6.10 of Lamb¹ and Fig. 2a of Wright²⁰). We have investigated patterns in the global distribution of sea-level pressure (SLP), surface-level air temperature (SLT), precipitation and SST in a 23-year integration of a version of the Oregon State University General Circulation Model (OSU GCM). This model has a resolution of 4° latitude and 5° longitude, has two layers in the atmosphere between the surface and 200 mbar with internally calculated cloudiness and radiation, and has two layers (of variable depth) in the ocean representing the mixed layer and the thermocline²¹. The atmosphere and ocean interact according to the conventional bulk formulae, with the sea-surface temperature internally predicted. The model exhibits secular trends in the computed SLP, SST and SLT due to an apparent overestimation of oceanic mixing in the tropics (W. L. Gates and G. L. Potter, personal communication); as a result the model's tropical SST is several degrees Celsius too cold. Since we are investigating interannual fluctuations in the computed fields we have removed the trends by subtracting a linear least-square fit of the monthly values at each grid point.

We have correlated annually averaged SLP at each grid point with that at 130° E, 2° S (Fig. 1a). Only regions with correlations significant at $\geq 95\%$ confidence level (two-tailed significance test) are shown. Comparable maps of pressure correlations based on observations have been given by Wright²⁰, Trenberth²² and Berlage²³. The results reported by Wright²⁰ are reproduced in Fig. 1b for comparison with Fig. 1a. The large region of positive correlation in the Indian Ocean and the complementary region of spatially coherent anti-correlation off the South American coast (Fig. 1a) are qualitatively consistent with Fig. 1b. Additionally, the model correctly reproduces the negative correlation region near the Bering Sea. Features of negative correlation adjacent to the Antarctic, seen in Fig. 1a, are similar to those of Trenberth²² and Berlage²³. The region of positive correlation extending north-west from North Africa in Fig. 1a is not found in the observations which generally show weak positive correlation over North Africa and negative correlation in the North Atlantic.

While Fig. 1 suggests that an essential aspect of the Southern Oscillation is being reproduced in the model, additional evidence for the internal physical consistency between the computed atmospheric and oceanic fields may be obtained by comparing with the empirical analyses of SLP, SLT and precipitation presented by Wright²⁰. He showed that annually averaged SLP at Darwin, Australia is positively correlated with SLT over the eastern equatorial Pacific. Figure 2 shows the correlation field

of annually averaged SLTs with SLP at 130° E, 2° S in the model. The large positive correlation in the eastern Pacific is consistent with that presented by Wright, although its meridional extent is smaller than in the observed field. Positive correlation indicates warmer temperature when pressure is high over Australia-Indonesia. Figure 2 also shows significant positive correlations across the northern subtropical Atlantic Ocean and the southern edge of Africa. The empirical results of Wright indicate weak positive correlations in these regions. Wright²⁰ has also shown that annually averaged SLP at Darwin, Australia is positively correlated with precipitation over the equatorial mid-Pacific. Figure 3 shows the correlation field of annually averaged precipitation with SLP at 130° E, 2° S. The positive correlation in the equatorial Pacific is consistent with that presented by Wright, although its longitudinal extent is smaller than in the observed field. Positive correlation indicates enhanced rainfall when pressure is higher at Australia-Indonesia. Figures 2 and 3 show that the response of surface temperature and precipitation to fluctuations in the trans-Pacific pressure gradient is simulated in the model consistent with observed behaviour of the Southern Oscillation.

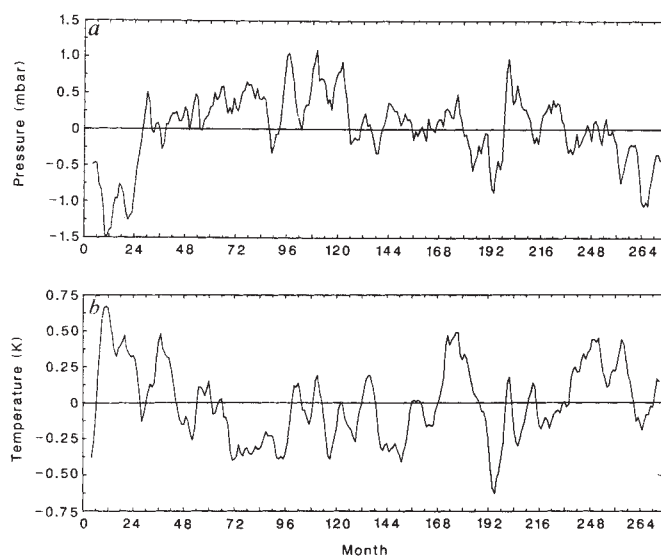


Fig. 4 a, OSU GCM Southern Oscillation Index shown is the monthly sea-level pressure anomaly at 115° W, 2° N minus that at 130° E, 2° S smoothed with a seven-month moving average. b, Anomaly of the computed SST for 115° W, 2° N, also smoothed with a seven-month moving average.

We have repeated the calculations of Figs 1–3 with several subsets of the model output and have found similar results in each case. It may also be noted here that if trends are not removed from the model output, as described earlier, the computed correlations and anti-correlations in the tropical core regions of Figs 1–3 are larger in magnitude as well as in spatial extent.

The Southern Oscillation Index is a measure of the see-saw of pressure between Indonesia and the eastern Pacific. We have calculated the OSU GCM Southern Oscillation Index as the difference of the pressure anomaly at 130° E, 2° S from that at 115° W, 2° N (the core grid points of the positive and negative correlation regions in Fig. 1a). This index (with seven-month smoothing) is presented in Fig. 4a. The magnitude of the fluctuations of the model Southern Oscillation Index is approximately a third to half that of the observed Index²⁰. This indicates that the simulation of Southern Oscillation in the model is weaker than the observed phenomenon. It has been noted that negative values of the Southern Oscillation Index correspond with positive SST anomalies in the eastern Pacific. Figure 4b shows the SST anomaly at 115° W, 2° N (with seven-month smoothing). The correlation coefficient between the curves in Fig. 4a and b is -0.40 (a correlation coefficient of absolute value ≥ 0.16 is significant at a 99% confidence level). Fig. 4 shows that the opposition of the fluctuations is approximately coincident for the first two-thirds of the model record but in the later part occurs with a lag of about one year (with SST leading the pressure index). The correlation coefficient between the two curves for the first 168 months is -0.56; it is -0.61 for the later portion with a lag of 13 months.

Although considerable progress has been made in the study of Southern Oscillation in regional models, its detection in a GCM simulation is expected to aid in understanding its mechanism

and its implications for global climate. Diagnostic studies of the outputs of multi-year integrations of this and other coupled GCMs may be expected to provide insight into the role of atmosphere-ocean coupling, the relationship between tropics and extratropics and the interactions of synoptic and seasonal fluctuations in the evolution of the Southern Oscillation phenomenon.

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Influence of solar variability on global sea surface temperatures

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Recent measurements¹ have shown that the total solar irradiance decreased at a rate of 0.019% per year between 1980 and 1985, and may still be decreasing. Presumably, this reflects a cyclical variation that may or may not be related to the well-known cycles of solar activity. Using data on globally averaged sea surface temperature (SST) over the past 120 yr², I show that the solar irradiance may have varied in phase with the 80–90 yr cycle represented by the envelope of the 11-yr solar-activity cycle. As the last peak of this cycle occurred in 1955–60, the next minimum should be reached about the end of the century, by which time the solar irradiance will be reduced from its peak value by ~1% if the present decay rate of 0.019% per year is typical.

In an earlier publication³ we drew attention to a correlation between the 12-month running mean height of the tropopause over two radiosonde stations in the western tropical Pacific Ocean and the annual mean sunspot number for the 22-yr period 1952–73. The correspondence had been noted earlier for shorter periods of time and for other tropical stations by other authors^{4–6}. We proposed at the time that the relationship could be explained by a small variation in solar irradiance accompanying the solar cycle. Such a variation would lead to corresponding small changes in average SST over the tropical oceans, and hence to changes in the latent-heat release due to deep convection, and ultimately to changes in tropospheric temperature and

in tropopause height. Although entirely speculative, the mechanism seemed plausible, and by the use of a simple model we estimated the peak-to-peak variation in solar irradiance needed to explain the observations as a few tenths of a per cent. The correlation was highly significant for the period 1952–69, but much weaker for the succeeding period 1970–80⁷.

The height of the tropical tropopause is at best an indirect indicator of SST over the tropical oceans, and a better parameter to use in studies of this kind would be the globally averaged SST based on direct measurements. Such a time series has been constructed^{2,8} back to 1854 from about 50 million non-duplicated observations with new corrections for pre-1942 SST data (C. K. Folland, personal communication) designed to eliminate as far as possible the effects of changing techniques and instruments over the years. As these corrections are comparable in magnitude with the long-term variations, and as the data are sparse in some latitudes, particularly in the southern hemisphere, the results need to be treated with some caution. The overall characteristics of the variation seem reasonably well established, however.

Figure 1 shows an 11-yr running average of the most recent version of the global SST variation (C. K. Folland, personal communication), expressed as departures from an arbitrary zero level, together with a similar 11-yr running average of the sunspot number. While far from identical, the two curves have enough points of similarity to lead one to speculate that a long-term variation in solar luminosity may have been a major factor influencing global mean temperatures in recent decades, and that this luminosity variation may have been positively correlated with the envelope of the 11-yr solar-activity cycle. This envelope has a quasi-periodicity of ~80–90 yr (the so-called Gleissberg cycle⁹), while the SST time series has a principal period of ~83 yr⁸.

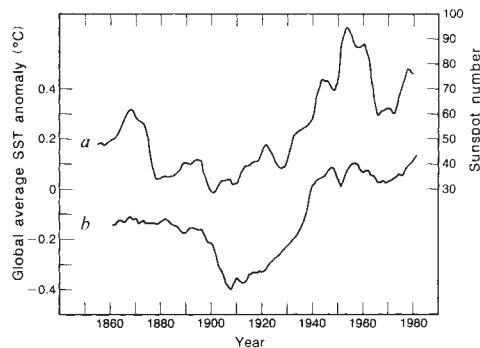


Fig. 1 The 11-yr running means of sunspot number (a), global mean SST anomalies (b).

Periods in the vicinity of 70–100 yr are ubiquitous in solar and solar-terrestrial physics, having been reported in measurements of the solar radius¹⁰, in historical records of auroral activity¹¹, in the oxygen isotope content of polar ice cores¹², and even in the thickness of Precambrian lake varves¹³, which are thought to reflect ancient solar activity. A positive correlation between solar luminosity and the intensity of solar activity was invoked by Eddy¹⁴ to explain the apparent coincidence between the Maunder minimum in solar activity and the peak of the Little Ice Age in Europe and North America, as well as similar coincidences in earlier times between various climate indicators and solar activity as revealed by the carbon-14 record. Recently Ribes *et al.*¹⁵ have also presented evidence that the solar radius was larger than usual during the Maunder minimum, which is consistent with Gilliland's¹⁰ finding that the long-term variation in solar radius was in antiphase with the Gleissberg cycle. There is thus a chain of circumstantial evidence suggesting that small cyclical changes in solar radius are accompanied by small and oppositely phased changes in the sun's luminosity, which in turn cause small variations in global mean temperatures.

In order to place this on a more quantitative footing, it is necessary to estimate the amplitude of the luminosity variation needed to produce the observed SST variation, assuming that it is due to solar variability alone. Many fairly simple numerical models of terrestrial climate have been developed in recent years^{16–20} in order to estimate the relative importance of solar and volcanic forcing, and to predict the impact of increasing atmospheric concentrations of CO₂ and other greenhouse gases. We have used the one-dimensional ocean thermal model of Hoffert *et al.*¹⁸ to estimate the thermal response of the upper ocean to a variation in solar constant of the form

$$S(t) = S_0[1 + \beta(N(t) - N_0)] \quad (1)$$

where $N(t)$ is the instantaneous value of the envelope of the sunspot number and S_0 and N_0 are reference values. The constant β is adjusted to give the best fit between observed and calculated SST.

Briefly, the model contains a 100-m deep mixed layer overlying the main 4,000-m deep ocean. Heat diffuses down into the ocean by eddy conduction, while cold water advects upward from below as part of a global thermohaline circulation driven by the sinking of dense surface water in the polar oceans. The model parameters used were essentially identical to those used by Hoffert *et al.*¹⁸.

Figure 2 shows the predicted SST variation for $\beta = 1.08 \times 10^{-4}$ superimposed on the annual values of the global mean SST, again expressed as departures from an arbitrary zero. This value of β gave the best fit to the amplitudes of the two curves, and the corresponding amplitude of the luminosity variation can be found from $\Delta S/S_0 \approx \beta \Delta N$. As ΔN from 1910 to 1960 is ~ 57 , the fractional range in luminosity is $\sim 0.6\%$. About 30 yr would be needed to produce such a change at the presently observed decay rate of 0.019% per year. In fact the change required ~ 50 yr, which is reasonably consistent.

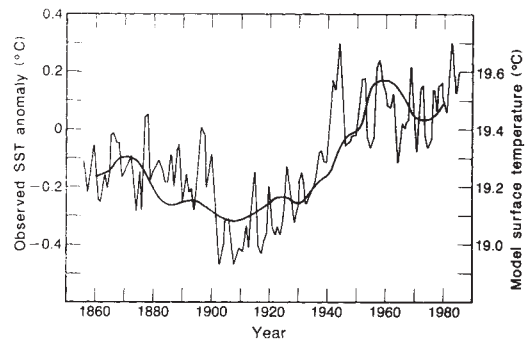


Fig. 2 Response of model surface temperature (right-hand scale) to solar luminosity variations proportional to the solar-cycle envelope (heavy curve), and annual variation in global mean anomaly of observed SST (left-hand scale).

The assumption that solar variability has been the only cause of global temperature changes is almost certainly unrealistic. Some models have predicted that the increasing atmospheric concentration of CO₂ should have produced a temperature change comparable with that observed since the pre-industrial era. Part of the rise seen between 1910 and 1960 could thus be attributed to the CO₂ effect, but it cannot account for the other features of the SST record. Trial calculations were made using the CO₂ parameterization of Hoffert *et al.*¹⁸, and assuming a 2.5°C increase in global mean equilibrium temperature for a doubling of atmospheric CO₂. The required range of variability of the solar constant was reduced from 0.6% to 0.4%, but the fit was somewhat less satisfactory than that shown in Fig. 2.

The secular variation in globally averaged SST over the past 130 yr has been found to show a certain amount of similarity to the corresponding variation of solar activity as revealed by the envelope of the 11-yr sunspot cycle. The intriguing possibility that the link might be provided by a small variation in the sun's luminosity keyed to the 80–90-yr Gleissberg cycle in solar activity has been explored with a simple ocean thermal model, and the range of variation needed has been found to be less than 1% over the 130-yr period. The implied rate of change in luminosity is consistent with the magnitude of the long-term decay that has been directly observed in recent years. Though entirely circumstantial and speculative, such clues to the causes of natural climate variability may prove valuable in future attempts to assess the climatic impact of man's activities. The need for a continuation of the current solar irradiance measurements over future decades is obvious.

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Lead $^{206}\text{Pb}/^{207}\text{Pb}$ isotope ratios in the atmosphere of North America as tracers of US and Canadian emissions

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Lead emitted into the environment (primarily by gasoline combustion and industrial processes) retains the isotopic composition of the ore from which it was derived. Therefore, there is the potential to distinguish sources of lead pollution in the atmosphere by examining the lead isotope ratios¹⁻⁴. Such a tracer of atmospheric lead would be valuable in air pollution transport studies, as the relatively long residence time of lead aerosols (~5-10 days), can result in its dispersal over thousands of kilometres. In practice, the situation is complicated by the wide, and frequently changing, variety of ore types used in gasoline additives and in smelting activities^{5,6}. This approach has generally only been successful in identifying local sources of pollution⁷⁻⁹, except where gasoline lead compositions have been deliberately altered¹⁰. We have discovered that the lead isotopic composition of atmospheric particulate matter in the eastern United States is distinctly different from that in eastern Canada, and that the composition is sufficiently constant between sites in each country over periods of several months to allow characteristic isotope ratios to be defined for each. This offers a new approach to examining transboundary pollution between the United States and Canada.

Samples of total atmospheric particulate matter were obtained from the filter archives of a number of institutes in eastern North America, and also from our own sampling. Measurement locations are shown in Fig. 1. All were from high-volume samplers operated at $\sim 1.13 \text{ m}^3 \text{ min}^{-1}$ over periods of one to two days. The filters used were either Whatman 41 cellulose or glass fibre. Portions of the filters were extracted into hydrochloric and nitric acids with the final solutions made up in 10% nitric acid. The $^{206}\text{Pb}/^{207}\text{Pb}$ ratios in the solutions were determined with an inductively-coupled argon plasma source mass spectrometer and normalized to an NBS.981 standard reference material. Total lead concentrations were determined with an inductively-coupled plasma optical spectrometer. Coefficients of variation of individual $^{206}\text{Pb}/^{207}\text{Pb}$ ratio measurements were typically $\sim 0.6\%$. There was insufficient sample to measure the ratios of the other natural isotopes of lead (^{204}Pb and ^{208}Pb). However, in the majority of lead ores, these ratios follow the same general trends as $^{206}\text{Pb}/^{207}\text{Pb}$ (ref. 11).

The results, divided into samples representing US and Canadian sources, are shown in Fig. 2. Each point is an average. Error bars represent the standard deviation of five replicate analyses. It is apparent that the $^{206}\text{Pb}/^{207}\text{Pb}$ ratios were consistently higher in the US samples. In the 1982-84 US city samples, the isotope ratios were very similar at all sites. There is some evidence that, for example, the Rock Island, Illinois ratios were lower than others, but no clear differences can be ascribed on the basis of the small number of samples. Between 1984 and 1986, the situation changed somewhat. The isotope ratios from Milwaukee and the four Illinois cities appeared to have increased slightly, whilst the ratios in Narragansett had clearly dropped. All these differences were much less than the large difference between US and southern Ontario values. An unusually high isotope ratio was recorded from one of the Chicago samples.

The Nanticoke, Ontario samples were collected from two sites on the north shore of Lake Erie, remote from heavily trafficked

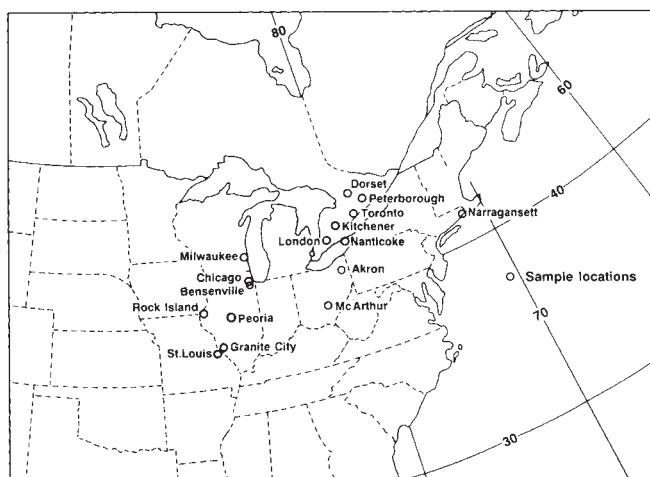


Fig. 1 The location of aerosol sampling sites in eastern North America. The Science Centre, City, Downsview, and Port Credit sites were all located in the Toronto area.

roads. Samples were selected from days on which the backward air mass trajectories showed transport of air from across the border. High isotope ratios were observed, although there was some scatter of results; in particular, five of the points were distinctly lower. This probably resulted from mixing with Canadian emissions of lower $^{206}\text{Pb}/^{207}\text{Pb}$ ratio. Apart from these outliers, the main body of data was quite uniform and consistent with the ratios observed in the US city samples, even though they were collected at an earlier date (1982/83). There were also two days with exceptionally high isotope ratios, similar to the high ratio recorded for one of the 1986 Chicago samples. The degree of consistency between sites can be seen in the ratios from samples collected on the same days at the two sites, which were 50km apart.

For the 1982-84 US city samples, we can calculate an overall mean $^{206}\text{Pb}/^{207}\text{Pb}$ ratio representing the isotopic 'signature' of eastern US lead emissions for that period. This has a value of 1.213 ± 0.008 (mean and standard deviation) for 25 data. In 1986, 14 data for the mid-west cities (those apart from Narragansett) gave a US isotope ratio of 1.221 ± 0.009 with the restriction that this may not be applicable to east coast emissions, which might be better represented by the Narragansett mean ratio of 1.196 ± 0.007 for nine data. It should be noted that, in terms of transboundary pollution, the mid-west area is of much greater significance due to the prevailing southerly to westerly air flow. Thus, the uncertainty related to the isotopic composition of east coast emissions is of limited concern.

In 1982-84, total lead concentrations for the US city samples varied from a mean of 73 ng m^{-3} for Narragansett to 437 ng m^{-3} for Granite City. In 1986, the mean lead concentrations were lower at all sites, perhaps due to the reduction in gasoline lead implemented that year. In Narragansett, the mean was just 22 ng m^{-3} , the lowest of all the sites, whilst Milwaukee had the highest mean of 178 ng m^{-3} . The Nanticoke mean was 46 ng m^{-3} in 1986 (no total Pb data available for earlier years).

Urban $^{206}\text{Pb}/^{207}\text{Pb}$ ratios for Ontario were obtained from two downtown sites ('City' and Science Centre) and a suburban location (Downsview) in Metropolitan Toronto, together with samples from the town centres of some larger southern Ontario communities (London, Kitchener, Peterborough and Port Credit). With the exception of Port Credit, these $^{206}\text{Pb}/^{207}\text{Pb}$ ratios were relatively constant between sites and over time. The ratios of the few samples collected at Nanticoke in 1982-83 during persistent north easterly winds (that is, air coming from Ontario) also corresponded well with these ratios. This indicates

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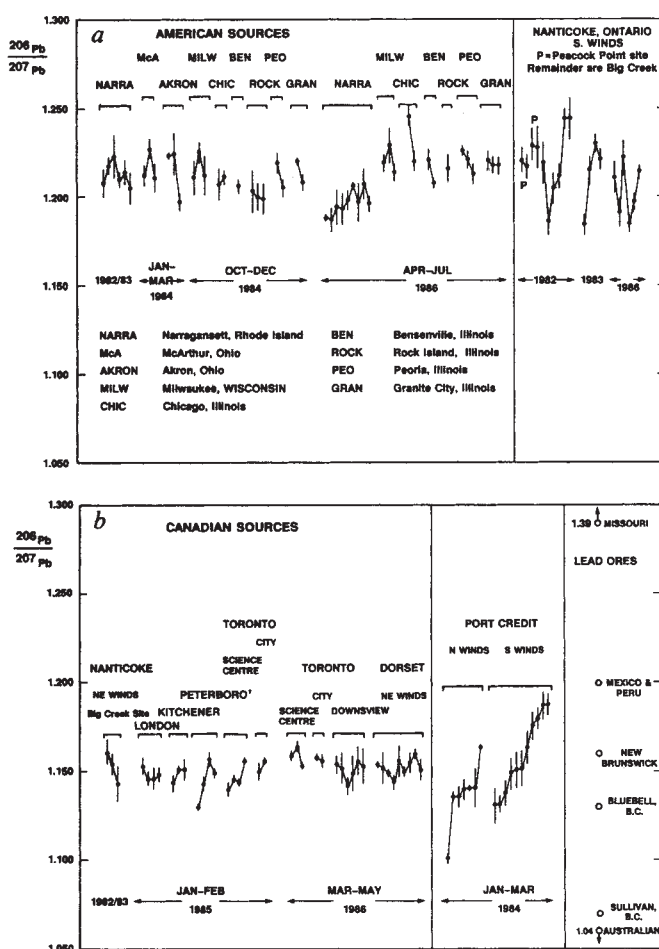


Fig. 2 Lead isotope ratios ($^{206}\text{Pb}/^{207}\text{Pb}$) in atmospheric particulate collected at: *a*, US urban sites and a rural location in Canada near the US border during air flow from the United States; *b*, Canadian (Ontario) sites. Part *b* also shows the isotope ratios of a few representative lead ores¹³. The data are plotted as the arithmetic mean (point) and standard deviation (bar) of five replicate determinations on the same sample.

that the southern Ontario area as a whole had a uniform isotopic lead composition, and also that the $^{206}\text{Pb}/^{207}\text{Pb}$ ratio had changed little between 1982 and 1986. There is evidence in the results from samples collected at the rural Dorset location that the isotopic composition observed in southern Ontario samples is characteristic of a large region of eastern Canada. Backward air trajectories for these samples originated in the Atlantic and travelled solely across Canadian territory before arriving at Dorset. The isotope ratios were all very close to those determined from the city locations in the same year (1986).

The Port Credit results show a wide spread of isotope ratios. This is understandable because the site is on the north shore of Lake Ontario, which receives either US air from the south or Canadian air from the north. Furthermore, a secondary lead smelter is located ~10 km to the north, which was probably responsible for the one unusually low isotope ratio. The same sample also contained the highest arsenic content of all the samples (G. Pringle, personal communication). To a lesser extent, the other samples collected during northerly flow may also have been affected by smelter emissions. We can determine isotope ratio 'signatures' for Canadian sources using the Ontario urban and Dorset results (excluding the Port Credit and Nanticoke data). The January–February 1985 mean ratio was 1.148 ± 0.007 for 17 data. In March–April 1986, it was 1.153 ± 0.005 for 20 data.

Table 1 $^{206}\text{Pb}/^{207}\text{Pb}$ ratios in selected major lead bearing ores

	$^{206}\text{Pb}/^{207}\text{Pb}$
Canadian automobile Pb sources	
North Star, B.C.	1.06
Sullivan, B.C.	1.07
Bluebell, B.C.	1.13
Monarch, B.C.	1.19
Brunswick no. 6, N.B.	1.16
Brunswick no. 12, N.B.	1.16
Reserve, N.B.	1.16
Keymet, N.B.	1.16
Manitouwadge, Ontario	0.92
Noranda Copper, Ontario	0.92
Miller Copper, Quebec	1.16
Federal Metals, Quebec	1.16
Buchans, Newfoundland	1.15
Flin Flon, Manitoba	1.01
Pine Point, N.W.T.	1.16
US automobile sources	
Durango, Mexico	1.20
Taxco, Mexico	1.19
Cerro de Pasco, Peru	1.20
Casapalea, Peru	1.20
San Cristobal, Peru	1.20
Mt Isa, Australia	1.04
Broken Hill, Australia	1.04
Mississippi Valley, Missouri	1.39

Data from ref. 1.

Lead concentrations at the Canadian urban sites ranged from 51 ng m^{-3} for Downsview to 287 ng m^{-3} for Kitchener. The mean for northerly winds at Port Credit was 170 ng m^{-3} and 114 ng m^{-3} for southerly winds. The mean for the Dorset samples was just 3.7 ng m^{-3} .

Why are there differences between isotope ratios in Canada and the United States? Despite the declining use of leaded gasoline in both countries¹², lead from automotive exhaust is still believed to be the major contributor to atmospheric lead concentrations. Most studies using bromine as a tracer of automotive lead show that, away from local industrial sources, lead and bromine are uniquely associated^{13,14}. Lead and bromine measurements from the Dorset site¹⁵ in 1984 indicated that the lead was overwhelmingly derived from gasoline. The observed $^{206}\text{Pb}/^{207}\text{Pb}$ ratios are therefore largely governed by the isotopic composition of the ores from which lead additives for gasoline are produced. Information on the origins of these lead ores is sparse, but private communications from various sectors of the petrochemical industry, and from Canadian Government sources, has led us to the following conclusions.

In Canada, lead additives have been produced exclusively by the Canadian divisions of the Dupont and Ethyl corporations until June 1985, when Dupont ceased production. Dupont obtained ores from New Brunswick, and Ethyl from British Columbia. Less than 1% of additive lead ore and leaded gasoline used in Canada was imported. Examination of the isotope ratios for selected major ores in Table 1 shows that the atmospheric lead isotope ratios in eastern Canada were consistent with the isotope ratios of the New Brunswick and Bluebell ores. The isotope ratios of the other Canadian ores shown in the Table are with one exception, no more than 1.16. Thus, the ratios in Canadian atmospheric particulate matter are likely to remain low, irrespective of changes in ore usage. Lead emissions from lead smelting operations should also contain lead with low isotope ratios assuming that the lead ores are obtained from similar sources. (Different isotope ratios, however, may be found in emissions from the smelting of other metal ores.)

In the United States, leaded additives are at present produced by Dupont and Ethyl. Prior to 1984, some was also manufactured

by Nalco. Ethyl imports ores from Mexico, Peru and Australia, and Dupont from various locations including New Brunswick. Before 1984, some Missouri ore with its anomalously high $^{206}\text{Pb}/^{207}\text{Pb}$ ratio was also used. On the basis of the isotope ratios in Table 1, the high US ratios observed in this study could be obtained from a mixture of Mexican, Peruvian, and Missouri leads, even though domestic ores are supposedly no longer used. Either minimal amounts of low isotope ratio Australian and Canadian ores were involved, or they were counterbalanced by similar quantities of domestic ores. One possibility is that there is a significant non-automotive (industrial) component to the atmospheric lead levels. In fact, there is active lead mining and smelting in the Mississippi-Missouri region. However, the uniformity of isotope ratios between sites does not support the notion of point-source contributions. Nevertheless, the occasional occurrences of exceptionally high isotope ratios which we observed might perhaps be so explained.

In conclusion, the United States and Canada emit lead to the environment that is mostly derived from separate geological sources, and therefore has different isotopic compositions. At locations away from local industrial sources in the Great Lakes/St Lawrence region, leaded gasoline is the primary source of lead in atmospheric aerosols. At such locations, it is therefore, in principle, possible to apportion the total airborne lead into two components, American and Canadian.

Whilst the pattern of lead ore usage in both countries is maintained, it can be expected that the eastern US $^{206}\text{Pb}/^{207}\text{Pb}$ ratios in aerosols will remain significantly above those of eastern Canada. However, the isotope ratios do vary somewhat from year to year. Therefore, in order to apportion sources accurately, it is necessary to determine source region isotopic compositions contemporaneously with observations at the receptor location.

We have applied this technique to samples collected at the aforementioned Dorset field site in central Ontario, about 200-km north of Toronto¹⁶, to discriminate US from Canadian emissions. Lead aerosol apportionments were estimated to be 55% Canadian and 43% American for the period October to December 1984, and 69% Canadian and 24% American for March to May 1986. A contribution from northern Canadian smelters (probably copper smelters) was also detected by the occurrence of very low isotope ratios, and estimated to have contributed 2% and 7% of the lead in the 1984 and 1986 studies, respectively. Insight is also being gained into the source of Arctic air pollution lead, using a similar approach on an hemispheric scale¹⁷.

We are indebted to Environment Canada for project support and to the Ontario Ministry of the Environment for access to their analytical facilities, and in particular to D. Boomer and M. Powell who developed the ICP/MS technique. We also thank all those who kindly contributed filter samples: P. Kelly and B. Foster of the Ontario Ministry of the Environment, K. Rahn and D. Lowenthal of the University of Rhode Island, G. Pringle of the University of Toronto, J. Chazin and J. Strauss of the University of Wisconsin and Wisconsin Department of Natural Resources, and D. Kolaz and T. Sweitzer of the Illinois Environmental Protection Agency.

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Variations of upwelling intensity recorded in varved sediment from the Gulf of California during the past 3,000 years

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Circulation in the Pacific Ocean is periodically affected by El Niño events which are a direct response to intensity changes in trade winds^{1,2}. The appearance of anomalously warm surface water in the eastern equatorial Pacific Ocean is one of the most spectacular manifestations of this event. Warm surface-water anomalies are also observed in the Northern Hemisphere along the Californian coast and in the Gulf of California. In the Gulf, strengthened trade winds induce upwelling during the winter, whereas in the summer, prevailing southerly winds provoke an input of warm water from the south³. We have sampled laminated sediments from the slopes of Guaymas Basin to reconstruct, with high resolution, variations of the hydrology of surface waters in the central part of the Gulf of California (sedimentation rate is of the order of 4 mm yr⁻¹ at the top and 1-2 mm yr⁻¹ down core). By analysing the oxygen isotope ratio of biogenic silica of diatoms⁴, we have recorded the surface-water temperature variations in the Guaymas Basin. The comparison of these fluctuations and variations of silicoflagellate abundance has allowed us to reconstruct changes in upwelling intensity during the past three thousand years. The present conditions of low upwelling activity are seen to be abnormal in comparison with those prevailing in the past.

The studied sediment cores come from the Guaymas Basin slope, one of the most productive areas of the Gulf of California (Fig. 1)^{5,6}. They have been collected from depths (630 m and 620 m respectively for E13 and E17) where dissolved O₂ concentration in bottom water is low enough to ensure preservation of the seasonal fluxes of sediment particulates⁷. Box core BAM80 E13 (24 cm long), was sampled at 4-mm intervals to provide a very detailed record of the past century. Analysis of ¹³⁷Cs was used to identify the 1964 horizon of maximum fallout⁸. In Kasten core BAM80 E17 (4-m long) the sampling interval is ~5 cm but not regular; varve counting was used to establish a chronology that spans over the past 3,000 yr. Each sample from E13 is representative of ~1.5 yr whereas each sample from E17 covers ~20 yr.

The oxygen isotope ratio of silica from diatoms was obtained following the procedure described in Labeyrie and Juillet⁹ and Juillet-Leclerc¹⁰; before extracting oxygen from silica, a treatment allows the control and estimation of the isotopically unstable fraction of the biogenous silica which provides a way to eliminate its effect on the isotopic composition of silica⁴. The isotope results have been plotted against age (Fig. 2 for BAM80 E13 and Fig. 3 for BAM80 E17).

Both E13 and E17 cores show oxygen isotope variations of silica on two different timescales: rapid variations are superimposed on a smoothed trend of the signal. E13 shows a regular decrease of $\delta^{18}\text{O}$, with an amplitude of 4‰ against SMOW (standard mean ocean water), from the end of the past century until now. The amplitude of the variations found in E17 over the past 100 yr is 3‰; the highest $\delta^{18}\text{O}$ values occur between 1,500 and 2,000 yr BP.

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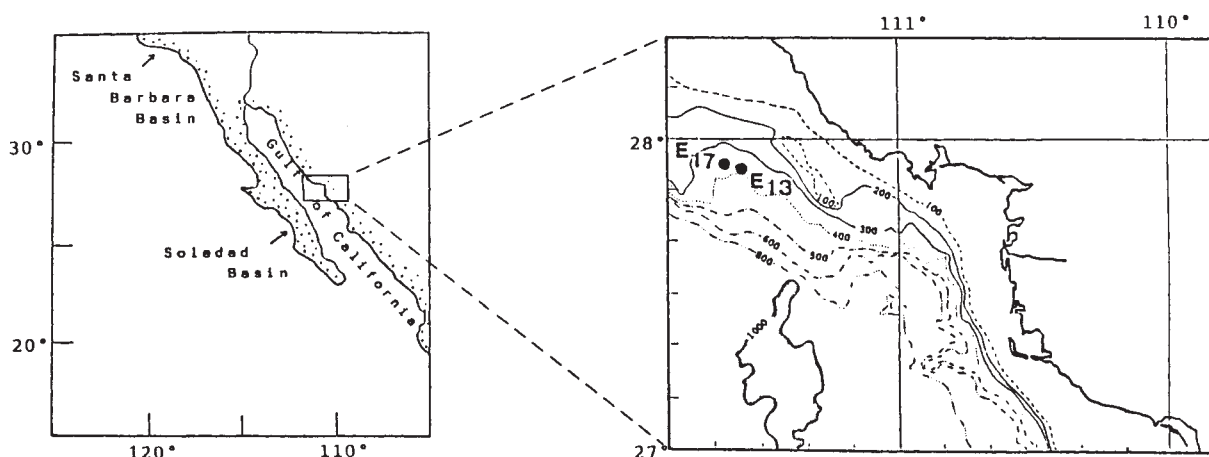


Fig. 1 Location of cores BAM80 E17 and BAM80 E13 in the Gulf of California.

The isotopic composition of silica is influenced by both temperature and the isotopic composition of water. Variations of isotopic composition of water may be due to global oceanic isotopic composition changes or local changes. We need not consider changes in global oceanic isotope ratio produced by melting or formation of continental ice sheets during the past 3,000 yr; isotope variations detected in diatom opal over this time span is a proxy indicator of surface-water mass variation in the Gulf of California.

For estimating local changes, we analysed the actual water masses in Guaymas Basin. Mean temperature, salinity and isotopic composition of water masses present in the Guaymas Basin or the water masses flowing into the Gulf are listed in Table 1. The average values have been calculated from direct measurements of isotope ratios from water sampled during May–June 1982 at different depths, and from the seasonal oceanic circulation fluctuations³. For the estimation of the isotopic composition of the Guaymas Basin water, we considered maximum possible changes in surface-water hydrology, to include the weak climatic variations which have occurred during the past 3,000 yr. After estimating the isotope effect of the dam construction of 1930 in the northern part of the Gulf, we concluded that fluvial input had a negligible isotope effect in Guaymas Basin during the period considered⁸. We observe in Table 1 that the maximum amplitude of $\delta^{18}\text{O}$ between the different water masses did not exceed 0.5‰; thus we cannot justify the variations of 3 or 4‰ of the isotopic composition of silica by a local change in isotopic composition of water. In this case, we consider that the variations of the isotopic composition from diatom opal is solely a function of temperature. For this study we adopted $0 \pm 0.3\%$ as an average value for isotopic composition of the water from Guaymas Basin, subject to the different effects noted in Table 1.

We have reconstituted the temperature variations in the Gulf of California using the calibration curve of $\delta^{18}\text{O}$ versus water⁴. The maximum temperature of 28 °C occurs at 1972–73 (Fig. 2); these years are marked by the occurrence of a strong El Niño¹². In the Gulf of California this event has provoked a rise in the sea level and temperature^{13–15}; the average measured temperature anomalies are ~ 3 or 4 °C, in good agreement with our results. The lowest temperature of E13 (15 °C) was observed at the end of the nineteenth century. The comparison of the temperature values obtained for the top of core E17, and the top of core E13, may be explained by the location of the core E17, closer to the upwelling centre¹⁶. But more probably this temperature difference indicates that a few centimetres (~ 10 cm) of sediment could be missing at the top of the Kasten core E17 (due to the coring technique). The total temperature amplitude observed in E17 for the past 3,000 yr is 8 °C. The highest temperature (17 °C) occurred at the end of the nineteenth century

and 3,000 yr ago. The time between these two warm events is characterized by cooler temperatures (13–14 °C) with only small variations, except between 3,000 and 2,000 yr BP, where a regular temperature drop of 6 °C is observed. We note that between 2,000 and 1,500 yr BP the minimum temperature (9 °C) was similar to values that have been measured during strong upwellings along the Californian coast¹⁷.

In the Gulf of California, two factors can explain these low temperatures: the occurrence of periods of intense upwelling activity or the input of cold water from the California Current which is a cold water mass flowing southward along the West coast of Baja California and entering the Gulf by passing around the tip of Baja California.

Floral analysis of samples from BAM80 E17 provides evidence of important upwelling activity fluctuations. Study of the silicoflagellate assemblages from plankton samples during two seasons (November 1980 and June 1982) in the Gulf of California^{18,19}, have shown that silicoflagellate distribution can serve as an ecological indicator: *Dictyocha messanensis* is considered to be representative of low productivity. The cold waters of the California Current appear rich in *Dictyocha messanensis* at the present time. In Fig. 3, we have plotted the variations of absolute abundance of *Dictyocha messanensis* versus sediment age next to the isotope curve. The two signals show that high $\delta^{18}\text{O}$ values correlate with low abundance of *Dictyocha messanensis* indicating that low temperatures are associated with high productivity. In the Guaymas Basin, productivity is essentially due to upwelling activity^{3,20,21}; the recorded temperature

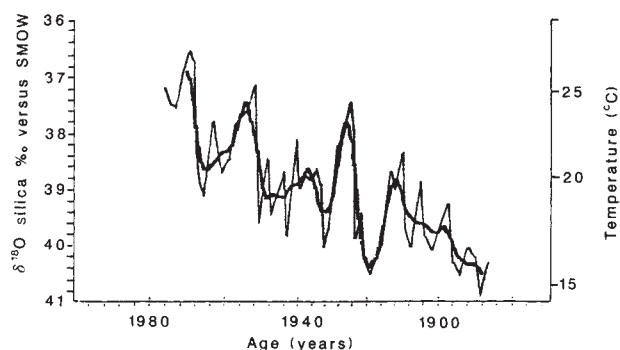


Fig. 2 Oxygen isotopic composition variations in diatom silica from BAM80 E13 versus age. The temperature scale corresponding with isotopic scale has been established from the relation:

$$t = 17.2 - 2.4(\delta_{\text{silica}} - \delta_{\text{water}} - 40) - 0.2(\delta_{\text{silica}} - \delta_{\text{water}} - 40)^2 \quad (\text{ref. 4})$$

The record has been smoothed by an 11-point least-square quadratic filter³.

Table 1 Oxygen isotope ratios of different water masses in the Gulf of California

	Temperature (°C)	Salinity (°)	Isotopic composition against SMOW*
Surface water (except during upwellings)	23	35.300	0.20
Upwelled water	13	35.000	-0.20
Equatorial water†	25	34.900	0
Californian Current water†	25	34.800	-0.10

The averages are calculated from measurements of water sampled during May-June 1982, taking account the seasonal variations.

* Average taking into account of the seasonal variations.

† Values at the mouth of the Gulf.

changes indicate upwelling intensity variations and not an intrusion of the California Current.

In the Gulf of California, the variations of upwelling intensity are directly linked with trade-wind fluctuations in the North Pacific Ocean. For instance, the isotope decrease found in 1972-73 sediments is associated with large changes in the trade wind regime. It was observed²²⁻²⁴ that the beginning of an El Niño event, trade winds are considerably reduced over all of the Pacific Ocean. This phenomenon is followed by a flow of warm water along the Peruvian coast which is propagated, along the coast into the Northern Hemisphere¹⁴; this wave leads to an increase of the sea surface temperature and sea-level in the Gulf. The isotope anomaly observed on E13, for the years 1972 and 1973, corresponds both to a decrease of trade-wind intensity and the input of warm water.

Northwesterly wind intensity has been decreasing steadily since the end of the nineteenth century. During the same time period large changes in the hydrology and climate have occurred. The isotope study of foraminifera from Santa Barbara Basin

reveals that upwelling triggered by trade winds has decreased between 1850 and now²⁵. By studying the abundance of pelagic fish debris, Soutar and Isaacs²⁶ have shown that the productivity sustained by intense upwellings and trade winds, decreased in the Santa Barbara Basin as well as in the Soledad Basin. Simultaneously general climatic manifestations have been observed: a mean global temperature rise⁷ associated with a glacial retreat in Mexico²⁸ as in numerous areas²⁹.

On a longer timescale, the past 3,000 yr, we note that in the Gulf the northwesterly winds attained their maximum intensity between 1,500 and 2,000 yr BP. This enhanced upwelling intensity is supported by the observations of Soutar and Isaacs³⁰ who have estimated that 1,500 yr ago, intense trade winds along the California coast, induced a productivity four or five times higher than that of the past 200 yr.

This isotope study of diatom opal from the Gulf of California provides evidence of fluctuations of surface-water temperature linked with trade-wind intensity over the North Pacific Ocean during the past 3,000 yr. The high-resolution record contained in the varved sequence allowed us to identify an abrupt oceanographic change since 1880. Therefore, the present oceanographic conditions are not representative of the past 3,000 yr. The same holds true for weather conditions. For instance, records of the mean global temperature of the past 134 yr show the highest values to occur after 1978²⁹. Now the Gulf of California is primarily under the influence of the North Equatorial Current¹⁵. However, trade-wind intensity was greater during the past 3,000 yr than during the past ten years.

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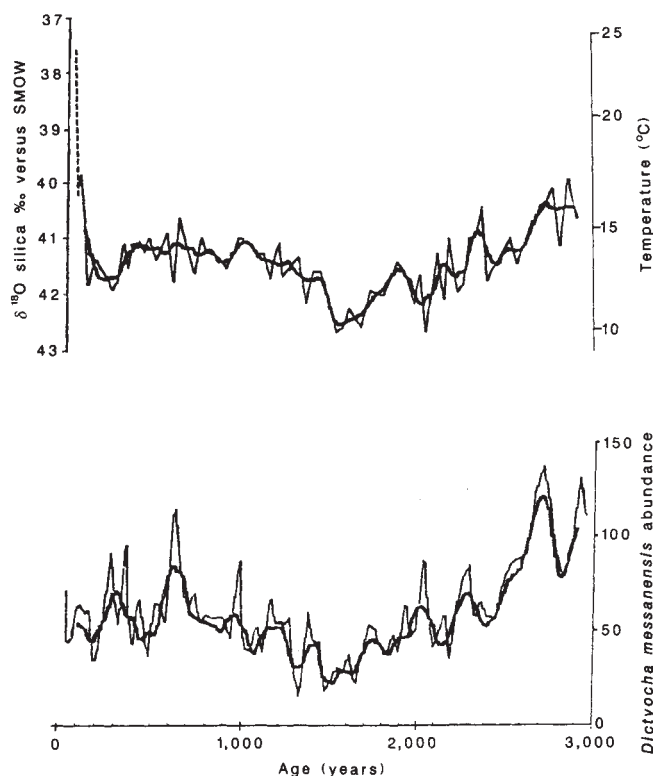


Fig. 3 Comparison of $\delta^{18}\text{O}$ variation in diatom silica and absolute abundance of *Dictyochoa messanensis*, versus age, in core BAM80 E17. The dotted line is the trend of the isotopic variations in BAM80 E13 samples. The record has been smoothed by a 21-point least-square quadratic filter³.

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Cataclysmic hydrothermal venting on the Juan de Fuca Ridge

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Serial observations of individual submarine hydrothermal vents¹ and the mapping of dilute hydrothermal plumes extending far downcurrent from vent fields²⁻⁴ indicate a stability of vent field fluid composition and mass flux on at least decadal time scales. The inherent episodicity of ridge-crest tectonic activity, however, suggests that discontinuous emissions of hydrothermal fluids also occurs. In support of this hypothesis we report here the discovery of a 700-m-thick, 20-km-diameter eddy-like 'megaplume' created by a brief but massive release of high-temperature hydrothermal fluids near 44°49' N, 130°14' W on the Juan de Fuca Ridge. The megaplume had a mean temperature anomaly of 0.12 °C and overlay compositionally distinct plumes emanating from an apparently steady-state vent field at the same location. The megaplume was formed in a few days yet equalled the annual output of between 200 and 2,000 high-temperature chimneys.

The plume features described here arose from a vent field discovered at the extreme northern end of the Southern Symmetrical Segment (SSS) of the Juan de Fuca Ridge during a systematic survey of hydrothermal activity along the ridge crest (ref. 5, E.T.B. *et al.* and G.J.M. *et al.*, in preparation). Detailed water column surveys during 17-23 1986 August using a deep-tow conductivity-temperature-depth (CTD)/transmissometer package⁶ mapped and sampled two plumes occupying the bottom 1,000 m of the water column and separated by a 50-m-layer of nearly ambient seawater (Fig. 1). The deep plume, with a maximum temperature anomaly (ΔT) of 0.06 °C, apparently originated in the axial valley of the ridge crest and extended off-axis to the southeast and along-axis to the north on the 27.68 potential density surface. Based on its asymmetrical distribution and its detection by a follow-up cast on 18 October the deep plume appears to be a steady-state feature typical of plumes emanating from other vent fields along the Juan de Fuca Ridge^{4,6-8}.

The upper plume is unique among published reports of neutrally-buoyant hydrothermal plumes. Rather than exhibiting the typically asymmetrical shape created by buoyant fluids continually injected into a laterally-flowing current^{4,6}, the megaplume geometry approximated a radially symmetric oblate spheroid indicative of a brief massive injection that created a 130-km³ plume of diluted hydrothermal fluids (Fig. 1). The temperature anomaly reached an extraordinary 0.28 °C at the megaplume centre, 700 m above the seafloor. The megaplume total excess heat

$$H = \rho C_p \sum \Delta T_i V_i$$

where specific heat $\rho C_p = 4.2 \times 10^6 \text{ J m}^{-3} (\text{°C})^{-1}$ and ΔT_i and V_i are the mean temperature anomaly and the volume between each ΔT contour. The heat anomaly thus calculated, $6.7 \times 10^{16} \text{ J}$, is a conservative estimate that includes only those fluids within the 0.04 °C ΔT contour.

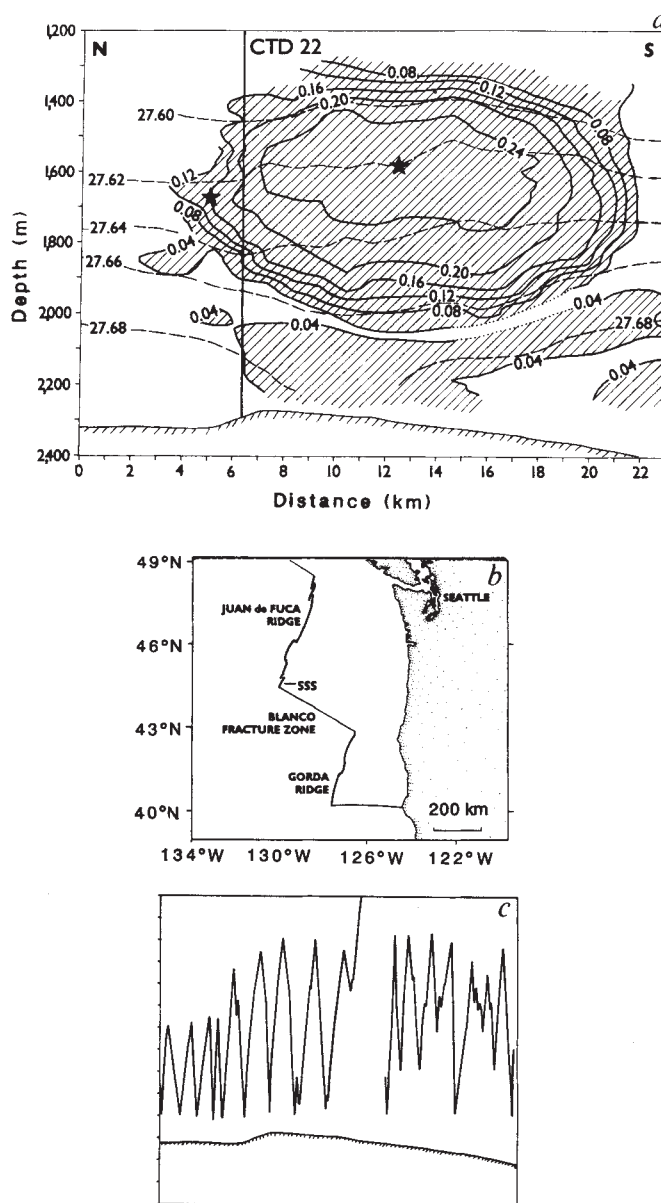


Fig. 1 a, North-south cross-section of temperature anomaly (°C) at the megaplume site. The temperature anomaly is the deviation along the potential temperature axis from the normally linear relationship between potential density and potential temperature⁸. It therefore represents the temperature elevation of plume water relative to ambient water at the same potential density. The 0.04 °C contour roughly defines the megaplume and the lower plume. Several cross-sections at different orientations showed the megaplume to be radially symmetric. Horizontal dashed lines connect surfaces of equal potential density (kg m^{-3}). Vertical line marks the position of CTD22 in the axial valley; stars mark the location of water samples filtered for mineralogic examination by scanning electron microscope and x-ray fluorescence. b, Location of the Southern Symmetrical Segment. c, CTD tow path used to construct the cross-section. Vertical and horizontal dimensions identical to a.

Mineralogic and hydrographic evidence support the geometric inference that the megaplume formed during a brief venting event. Large (5-130 μm) grains of the hydrothermal minerals anhydrite, chalcocopyrite and amorphous silica were common in two filtered samples from the centre and edge of the megaplume at a depth of ~1,600 m (E.T.B. *et al.*, in preparation). Because the large anhydrite grains could settle 70-200 m d⁻¹, the megaplume must have been no more than several

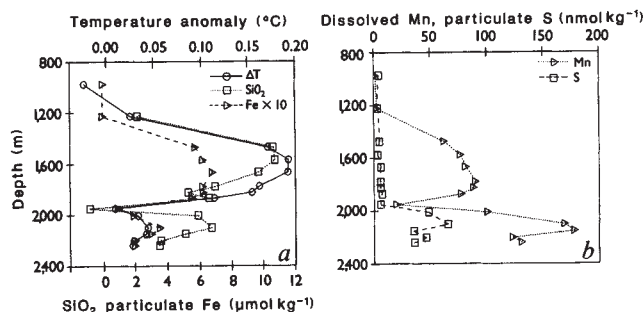


Fig. 2 Vertical profiles of selected hydrothermal constituents. *a*, Constituents enriched in the megaplume; *b*, constituents enriched in the lower plume. Samples collected by CTD22 (~0100 UTC, 20 August). Particulate chemistry was determined by energy dispersive X-ray fluorescence spectrometry using thin-film procedures (G.J.M. *et al.*, in preparation). Dissolved Mn was determined by flameless atomic absorption spectrometry after Klinkhammer¹⁸. Silicate was determined spectrophotometrically on-board¹⁹. The silicate anomaly is the raw value minus the regional silicate profile. Note that Fe is plotted at 10× its true value (maximum Fe concentration = 0.69 μmol kg⁻¹) to facilitate comparison with other profiles.

days old when the particles were collected (~17:00–24:00 UTC 21 August). Anhydrite grains at the megaplume edge were consistently in a more advanced stage of dissolution than those at the centre, implying that radial expansion took place over a period of several days rather than instantly. The vertical and horizontal dimensions of the megaplume remained constant during our survey, so expansion, and presumably venting, had diminished or ceased at the time of its discovery. No trace of the megaplume was found on 18 October, when we reoccupied the station at 44°49' N, 130°14' W.

Vertical profiles of conservative and nonconservative constituents alike describe two plumes with distinctly different compositions. The megaplume was enriched in heat, dissolved SiO₂ and Fe (98% particulate, 2% dissolved); the lower plume in Mn (98% dissolved, 2% particulate) and particulate S (Fig. 2). Particulate Fe, primarily in the form of sub-micrometre-sized amorphous oxide precipitates, constituted about 40% by weight of the megaplume solids, compared with only about 25% or less in the lower plume and other deep plumes on the Juan de Fuca Ridge (ref. 4 and G.J.M. *et al.* in preparation). The extraordinarily high Fe concentration resulted in an Fe/Mn molar ratio of 9.7, about four times higher than in either the lower plume or the plume from the vent fields near 44°40' N on this ridge segment⁴, where vent fluids with exceptionally high Fe concentrations (and Fe/Mn ratios of 4.0–5.2) have been sampled⁹.

What kind of tectonic process could release such an enormous quantity of heat and chemicals from the crust in only a few days? Several points argue against an explosive event. The momentum and buoyancy inputs likely associated with an explosion would have driven the megaplume much higher in the water column than was observed. Cross-plume differences in anhydrite dissolution, and the absence of recognizable pyroclastic fragments in the filtered water samples, also support a non-explosive origin.

If we reject an explosive event, then we must postulate the sudden liberation of a vast quantity of crustal hydrothermal fluid, or the sudden seawater flooding of an extensive surface of very hot rock. About 0.065 km³ of 350 °C fluid must mix with ambient near-bottom seawater ($T = 1.8$ °C) to produce the mean temperature anomaly (0.12 °C) of the megaplume. This volume represents fluid from 0.65 to 0.16 km³ of crust, based on porosities of 10–40%^{10–12}. Alternatively, the excess heat contained in the vent fluids may have been extracted from the sudden cooling of a basalt extrusion. Lava flood plains and

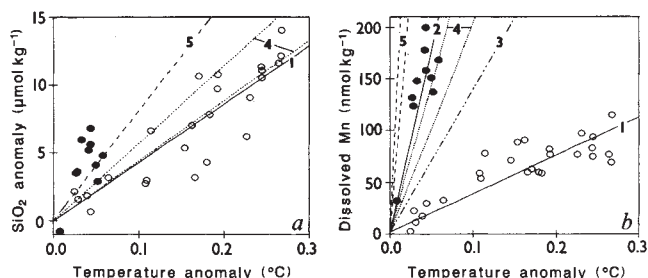


Fig. 3 Plots of temperature anomaly against SiO₂ (*a*) and dissolved Mn (*b*) in the megaplume (○) and the lower plume (●). Mass/heat correlations are 42.9 μmol cal⁻¹ ($r^2 = 0.79$) for SiO₂ and 0.37 nmol cal⁻¹ ($r^2 = 0.68$) for Mn in the megaplume (line 1), and 3.5 nmol cal⁻¹ ($r^2 = 0.90$) for Mn in the lower plume (line 2). These correlations are compared with the mass/heat correlations for SiO₂ and Mn in the neutrally-buoyant plume emanating from the 44°40' N site⁶ (line 3) and to back-extrapolations for the range of vent fluids at 21° N on the East Pacific Rise¹⁶ (exit temperature 350 °C) (line 4) and near 44°40' N on the SSS⁹ (exit temperature 285 °C) (line 5). All regressions have been forced through an intercept representing ambient seawater.

constructional volcanic features are common on this ridge segment¹³. The heat extracted from a given mass of molten basalt is the volume specific heat lost during cooling (3.8 J cm⁻³ deg⁻¹) and the latent heat lost during crystallization (10³ J cm⁻³); a 1,200 °C cooling of basalt liberates 5.6 × 10³ J cm⁻³. The megaplume excess heat thus represents a 1,200 °C cooling of 0.012 km³ of basalt, or a proportionally greater volume cooled less. This rock volume roughly equals the long-term annual average of crustal accretion to the 55-km-long SSS based on a full-rate spreading of 6 cm yr⁻¹ and an axial crustal thickness of 2.5 km (ref. 14).

The importance of instantaneous heating is conjectural, but the high Fe/Mn ratio in the megaplume is consistent with formation by a seawater-dominated hydrothermal system. Experimental alteration of basalt at high temperatures and pressures¹⁵ shows that the Fe/Mn ratio of hydrothermal fluids increases sharply as the water/rock ratio increases.

An intriguing hypothesis that incorporates some features of both fluid release and instantaneous heating is that the megaplume was the hydrothermal expression of an incremental sea-floor-spreading event. A fissuring of the axial valley floor by a dike intrusion and subsequent surface lava flows might release a large volume of fluid from the hydrothermal plumbing system as well as provide an extensive surface of molten rock for heating seawater. Baker *et al.* (in preparation) examined the likelihood of fissure vents giving rise to the observed plume by devising an enthalpy-conserving plume model in which the source was linearly distributed. Assuming an exit temperature of 350 °C and an ambient seawater salinity, the observed rise height of the megaplume requires a source heat flux of about 3.6 × 10⁸ J s⁻¹ per metre of fissure length. Dividing this heat flux into the heat anomaly of the megaplume yields a source strength of 2,200 m-days. Thus a 0.5 km-long fissure could create the megaplume in about four days. The fissure width depends on the fluid exit velocity, and would be <0.25 m if exit velocities exceeded 1 m s⁻¹.

Mass/heat correlations in the megaplume indicate a variable extraction efficiency for different constituents with respect to heat (Fig. 3). For SiO₂, the megaplume correlation falls at the low end of vent fluid data from 21° N¹⁶ or the vent field near 44°40' N on the SSS⁹, about 25 km south of the megaplume site. The lower plume correlation appears to differ, but the line is not well constrained. For Mn, the megaplume correlation is substantially below that found for vent fluids^{9,16}, the plume from the 44°40' N vent field⁶, or the lower plume, suggesting that Mn

was much less efficiently extracted from the crust than SiO_2 during the megaplume formation. Extrapolations from the mass/heat correlations and knowledge of the total excess heat in the megaplume allow comparison of the megaplume flux with that of a 350°C black smoker compositionally similar to those at 21°N^{16} and with a mass flux of $0.5\text{--}10\text{ kg s}^{-1}$ (ref. 17). The megaplume load of SiO_2 ($5.6 \times 10^8\text{ mol}$) and Fe ($5.7 \times 10^7\text{ mol}$) represents the annual output of about 2,000–200 chimneys, the same as for heat. The Mn load ($7 \times 10^6\text{ mol}$) represents only one-tenth as many chimneys.

The importance of massive, sporadic hydrothermal events ultimately depends on their frequency and their link to ridge-crest tectonic processes. If a basaltic intrusion of only 0.01 km^3 is sufficient to create a megaplume, hundreds to thousands of such plumes may be blossoming each year along the global ridge system. In addition to adding a new dimension to ideas about the exchange of heat and chemicals between the crust and seawater, sporadic plumes may serve as indicators of contemporary axial tectonic events and as natural tracers of the Lagrangian velocity field in the deep ocean. These observations thus demonstrate the need for ridge-crest observational strategies that will integrate time-dependent measurements of

both the tectonic causes and the hydrothermal-fluid effects of seafloor spreading.

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Inhibition of the firing of vasopressin neurons by atriopeptin

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Atriopeptin, the atrial natriuretic peptide, is a circulating hormone that is released from the atria of mammalian hearts in response to volume expansion and acts upon the kidneys, adrenal glands and vasculature to regulate fluid and electrolyte homeostasis¹. Atriopeptin is also present in the brain of the rat^{2–5}. Atriopeptin immunoreactive cell bodies and fibres are found in many areas known to be involved in the central regulation of the cardiovascular system, suggesting that it may be a neuromediator in the central control of fluid and electrolyte balance^{6–8}. The paraventricular nucleus of the hypothalamus, which contains the cell bodies of neurons that secrete vasopressin from the posterior pituitary gland, receives a dense innervation from atriopeptin-like immunoreactive fibres^{6,8–11}. We have studied the effect of atriopeptin on the electrical activity of single neurons in the paraventricular nucleus of anaesthetized rats and found that atriopeptin is a potent inhibitor of putative vasopressin neurons. Atriopeptin, which has systemic actions that oppose those of vasopressin, may act as a neuromodulator in the brain to prevent vasopressin secretion.

Atriopeptin immunoreactive fibres innervate the entire paraventricular nucleus. The parvocellular parts of the nucleus, concerned with control of the anterior pituitary gland and the autonomic nervous system, are most densely innervated^{11–14}. However, there are many immunoreactive fibres and terminals found in the posterior magnocellular portion of the nucleus, which contains cell bodies of neurons that secrete vasopressin and oxytocin from their terminals in the posterior pituitary gland^{11,13,14}. A large component of the atriopeptin-immunoreactive innervation of the paraventricular nucleus arises from a cluster of neurons near the anteroventral tip of the third ventricle, in the 'AV3V' region that is thought to be important in the

regulation of electrolyte homeostasis and vasopressin secretion^{7,15}. As recent studies have reported that application of atriopeptin can alter the firing of hypothalamic and septal neurons^{16,17}, we sought to determine its effects on the firing of vasopressin neurons.

Multibarrel micropipettes were used to record the activity of paraventricular neurons and to inject peptide solutions. The barrels of the injection pipette were filled with solutions of synthetic atriopeptin III (residues 103–126 of the prohormone) and des-[Gly 18 Leu 17] atriopeptin III-amide, an analogue that has no detectable activity in smooth-muscle bioassays, dissolved in 0.9% saline at concentrations of $10\text{ }\mu\text{g ml}^{-3}$ ($3.7\text{ }\mu\text{M}$, $n=20$) or $100\text{ }\mu\text{g ml}^{-1}$ ($37\text{ }\mu\text{M}$, $n=3$). The region of the paraventricular nucleus was explored in urethane anaesthetized rats that had been deprived of water for 24–48 h before surgery. We categorized neurons with periods of 10–20 Hz activity alternating with periods of silence as phasic, an activity pattern that has been closely associated with vasopressin neurons^{18–20}. As an additional criterion, we tested for rapid and reversible inhibition of firing during increases in blood pressure produced by infusion of pressor agents, another well-established property of vasopressin neurons¹⁸. Peptide solutions were applied using short pulses of air pressure, adjusted so that each pulse delivered 30–500 pl of solution. Changes in spontaneous neural activity produced by the peptides were assessed by comparing the mean firing rates and, for phasic neurons, the interburst intervals of the units; both of these properties are closely correlated with the secretion of vasopressin from the posterior pituitary gland.

A total of 23 spontaneously active units in the region of the paraventricular nucleus were examined for alterations in activity produced by atriopeptin. Thirteen of these units had a typical phasic pattern of activity. Nine of these phasic units were tested for their response to increased blood pressure; all of them were inhibited by the intravenous injection of phenylephrine, a vasoconstrictor that elevates arterial blood pressure. The inactive analogue of atriopeptin, applied in quantities of 1.5–840 fmol before the active peptide, had no effect on the activity of any of the units tested.

Two types of responses were observed following the application of atriopeptin to phasic units. Four phasic units, tested with 1.3–78 fmol of atriopeptin, showed a pronounced prolongation of the interval between phasic bursts with little change in mean activity during the bursts. This effect was gradual in onset, occurring over several minutes, and persisted for 240–3,200 s.

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Fig. 1 *a*, Continuous time histogram of the spontaneous activity of a single phasically active neuron in the posterior magnocellular part of the paraventricular nucleus of the hypothalamus (see Fig. 2). The inactive analogue of atriopeptin, applied near the beginning of the record in quantities ranging from 1.0 to 27 fmol ('Control') had no effect on the activity of the unit. The small decreases in firing associated with the lower atriopeptin (AP) doses (0.2 and 0.8 fmol) were probably within the normal range of variability of neuronal firing (note that the decrease in firing actually began about 40 s before the 0.8-fmol dose), as there was no effect at the 4.2 fmol dose. The overall increase in burst duration which occurred during the course of the experiment was within the range of activity that we typically found during prolonged recording sessions (more than two hours in this case). By contrast, application of 15 fmol of atriopeptin produced profound inhibition of phasic activity beginning at about 120 s and continuing with nearly complete absence of firing for a period of ~1,300 s, followed by the abrupt return of neuronal firing to baseline levels. This pattern of activity was only seen following the application of atriopeptin. No variation in the waveform of the action potential was observed at any time. This unit was inhibited by a brief period of elevated blood pressure following the intravenous injection of phenylephrine (PE). *b*, Interspike interval histogram for the same unit as in *a*, performed for 300 s during the control period. There is a peak interspike interval at about 50 ms, corresponding to a peak firing rate of 20 Hz during bursting. The bar at the far right shows the number of periods during which the unit did not fire for 1 s or more. This bimodal firing distribution is typical of vasopressin neurons¹⁸. Scale for both panels is 0–50 action potentials per bin. Bin width for *a* 4 s; for *b*, 5 ms.

Methods. Male Sprague-Dawley rats (250–300 g) deprived of water for 24–48 h were anaesthetized with α -chloralose, 75 mg per kg ($n=6$) or urethane 1.1 g per kg ($n=12$) and placed in a stereotaxic apparatus. A femoral artery catheter was used to monitor blood pressure and heart rate. Triple-barrel glass micropipettes (inner tip diameters 3–6 μ m) were used alone, or glued to a fine single-barrel recording pipette (tip inner diameter <1 μ m), to record electrical activity of paraventricular neurons either from a dorsal ($n=5$) or a transphenoidal ($n=13$) approach. Recording barrels were filled with 3M NaCl, and action potentials were amplified, monitored and discriminated using conventional means. Units which showed alterations in action potential waveform during application of the peptides were discarded. Continuous time histograms were generated with an Apple IIe computer using an RC Electronics program. Peptide solutions were applied using short pulses of air pressure (30–45 p.s.i., 5–50 ms, 1 Hz) delivered by a solenoid valve driven by a Grass stimulator. The volume ejected by these pulses was determined at the conclusion of each experiment by direct observation of the movement of the fluid meniscus in the pipette. Phenylephrine (2 μ g per kg) was injected through a femoral venous catheter to test the response of the unit to brief elevation of blood pressure.

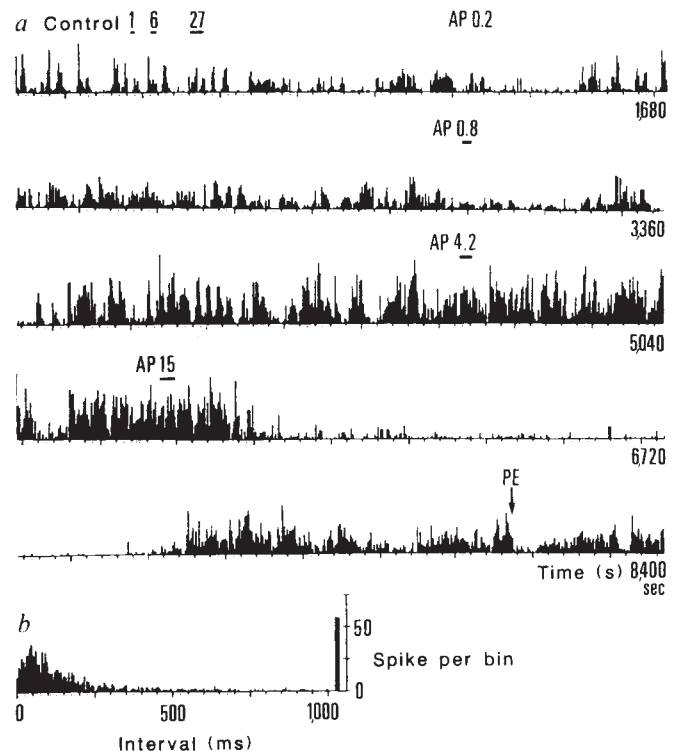
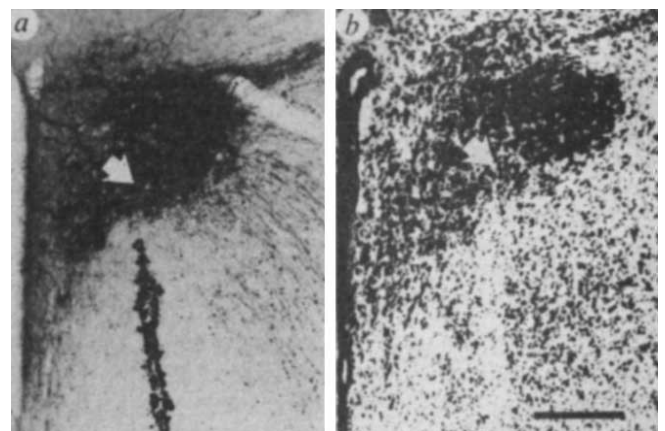


Fig. 2. Location of the recording site of the unit whose activity is charted in Fig. 1. *a*, Coronal section through the posterior magnocellular division of the paraventricular nucleus of the hypothalamus, stained immunohistochemically for arginine vasopressin; *b*, caudally adjacent section stained with thionin. Scale bar, 200 μ m. The large electrode track ventral to the nucleus was produced by the multibarrel injection pipette; the tip of the fine recording pipette extended about 100 μ m further into the vasopressin cell group (white arrows).

Methods. After recordings were made, animals were perfused through the heart with 10% buffered formalin, the brains were removed and 50 μ m sections were cut on a freezing microtome. Sections were incubated for one hour at room temperature in 0.1 M phosphate buffer, 0.9% NaCl, pH 7.5, containing 3% normal goat serum (PBS-G) and 0.25% Triton X-100; then overnight at 4 °C in rabbit anti-arginine-vasopressin antiserum (Immunonuclear) diluted 1:500 in PBS-G, then for 1 h at room temperature in horseradish peroxidase conjugated goat anti-rabbit immunoglobulin G antiserum (Tago 1:50 in PBS-G), followed by 10 min in 0.01% hydrogen and 0.05% diaminobenzidine in phosphate buffer. Another series of sections was stained with 0.01% thionin.



In the remaining nine cases, application of atriopeptin produced a long interval with no phasic bursts (mean duration 832 ± 176 s, range 160–1,680 s) followed by a rapid return to the baseline activity (Fig. 1). The time of onset of this effect was variable (mean 83 ± 25 s, range 4–160 s), probably reflecting the relative positions of the recording electrode, injection pipette and phasic neuron. In seven cases, atriopeptin was applied in gradually increasing quantities, so that the threshold for action of the peptide could be assessed. The smaller quantities tested typically produced little or no change in activity, whereas relatively small increments in dosage often produced a maximal response (Fig.

1). The mean apparent threshold for inhibition in these seven experiments was 5.7 ± 1.8 fmol (range 0.7–15 fmol).

Ten additional spontaneously active, non-phasic units were tested; eight of these were tested with intravenous phenylephrine, and only two were inhibited. Neither atriopeptin nor the inactive analogue altered the spontaneous activity of any of these non-phasic neurons in doses from 12 to 175 fmol.

The locations of all recording sites were determined in sections stained immunohistochemically for vasopressin. All 13 of the phasic units were in the vasopressin cell group in the posterior magnocellular paraventricular subnucleus (Fig. 2). All of the

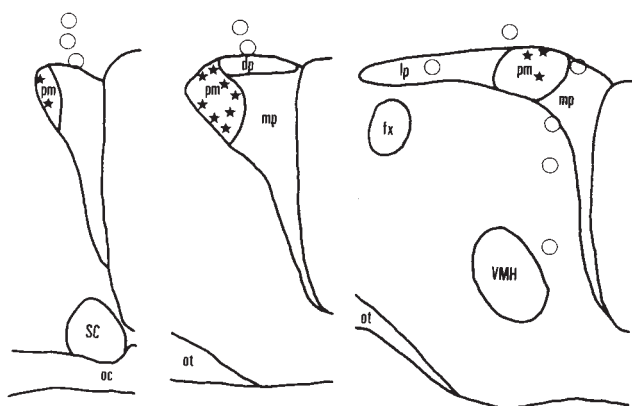


Fig. 3. Diagram summarizing the anatomical distribution of the units tested. Subnuclei of the paraventricular nucleus are shown at three levels from rostral (left) to caudal (right). ★, Recording site for phasic units; ○ non-phasic unit. Application of atriopeptin inhibited all the phasic units, but did not affect the activity of the non-phasic ones. Abbreviations: fx, fornix; SC, suprachiasmatic nucleus; pm, posterior magnocellular paraventricular nucleus; lp, lateral parvocellular paraventricular nucleus; dp, dorsal parvocellular paraventricular nucleus; VMH, ventromedial nucleus of the hypothalamus.

non-phasic units were in the parvocellular regions of the paraventricular nucleus or outside the borders of the nucleus (Fig. 3).

Our results indicate that application of femtomole doses of atriopeptin inhibits phasically firing, baroreponsive neurons in the posterior magnocellular paraventricular subnucleus. Recordings *in vivo* of paraventricular magnocellular neurons (identified by antidromic activation from the posterior pituitary gland) show that phasic firing is a unique property of neurons that respond to stimuli that cause vasopressin secretion, such as haemorrhage or an increase in plasma osmolality^{18,19}. However, the actual demonstration that phasic paraventricular neurons contain vasopressin has been accomplished by intracellular recording and marking of neurons only in the slice preparation²¹. In that study, 9 of 11 phasic cells in the paraventricular nucleus were vasopressin-neurophysin immunoreactive. Neither of the remaining cells were stained by an antiserum against oxytocin-neurophysin, suggesting that the absence of vasopressin immunostaining may have been a technical artefact. In any case, the combined electrophysiological and immunohistochemical criteria we used ensure that at least 80–90%, if not all, of the atriopeptin-responsive phasic neurons in our sample contained vasopressin.

Our results are consistent with previous studies showing that intracerebroventricular or intravenous injections of atriopeptin can suppress basal and dehydration- or haemorrhage-stimulated secretion of vasopressin^{22–24}. However, these studies do not address the issue of the locus of the action of atriopeptin. In our experiments, the time course of the inhibitory effect of atriopeptin on putative vasopressin neurons, and the small quantities of the peptide required, indicate that the response is mediated in the paraventricular nucleus. This implies the existence of neural receptors for atriopeptin either on the vasopressin neurons themselves or on nearby interneurons providing synaptic input to them. Thus atriopeptin released from nerve terminals innervating the paraventricular nucleus may have a physiological function in the control of vasopressin secretion, consistent with the systemic function of atriopeptin as a hormone regulating fluid and electrolyte homeostasis.

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Failure of familial Alzheimer's disease to segregate with the A4-amyloid gene in several European families

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The gene coding for the amyloid protein, a component of neuritic plaques found in brain tissue from patients with Alzheimer's disease, has been localized to chromosome 21, and neighbouring polymorphic DNA markers segregate with Alzheimer's disease in several large families. These data, and the association of Alzheimer's disease with Down's syndrome, suggest that overproduction of the amyloid protein, or production of an abnormal variant of the protein, may be the underlying pathological change causing Alzheimer's disease. We have identified a restriction fragment length polymorphism of the A4-amyloid gene, and find recombinants in two Alzheimer's disease families between Alzheimer's disease and the A4-amyloid locus. This demonstrates that the gene for plaque core A4-amyloid cannot be the locus of a defect causing Alzheimer's disease in these families. These data indicate that alterations in the plaque core amyloid gene cannot explain the molecular pathology for all cases of Alzheimer's disease.

Alzheimer's disease is the most common form of dementia, affecting as much as 5% of the population over 65 (ref. 1). It

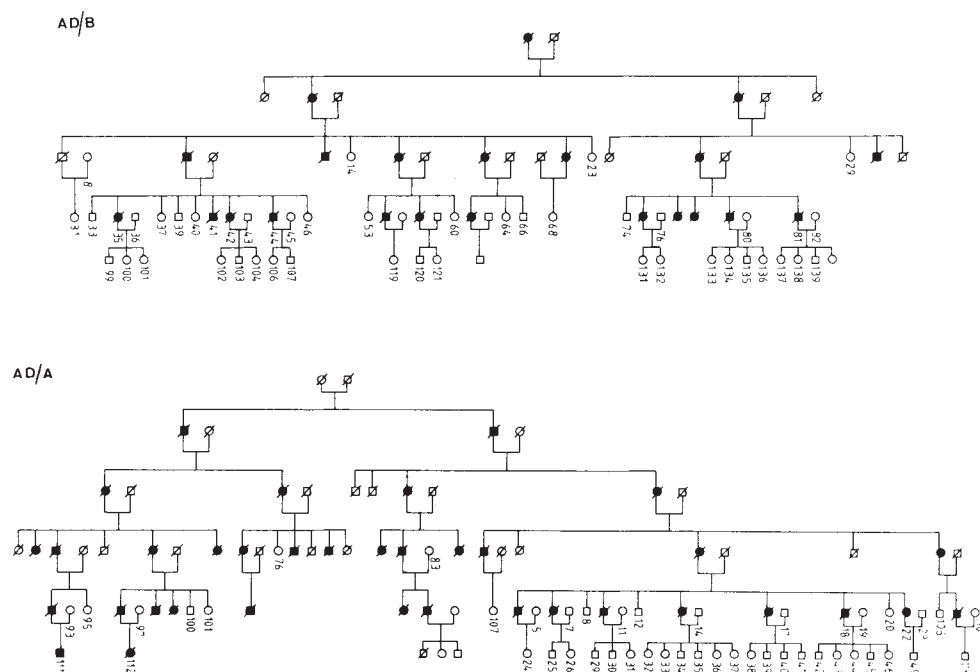


Fig. 1 Two large pedigrees with early onset Alzheimer's disease. Symbols: circle, female; square, male; diagonal line, deceased; filled symbol, affected individual. The numbers refer to the cases available for genetic linkage analysis. Family AD/A includes 36 Alzheimer cases in 6 generations of which 10 have been histopathologically confirmed. Mean age of onset was 33 ± 4 years. Family AD/B includes 22 Alzheimer cases in five generations of which four have been histopathologically confirmed. Mean age of onset was 34 ± 2 years.

is characterized by the presence of large numbers of neuritic plaques and neurofibrillary tangles, particularly in the hippocampus and the association cortex². The pathogenesis is not understood, although two groups are at high risk of developing Alzheimer's disease: those with a family history of the disease^{3,4} and persons with Down's syndrome⁵. In some pedigrees Alzheimer's disease segregates as an autosomal dominant disorder⁶⁻⁸. In four such pedigrees, molecular genetic linkage analyses have indicated a disease locus on chromosome 21 in the region of 21q11.2 $\rightarrow$ 21q22.2 (ref. 9), closer to the centromere than the region 21q22 associated with Down's syndrome¹⁰.

Recently the amyloid protein has been isolated from the Alzheimer's disease cerebral vessels (β -amyloid)¹¹ and plaque core (A4-amyloid)¹²⁻¹⁴ which have very similar amino-acid

sequences and are antigenically related^{15,16}. Oligonucleotides corresponding to a part of the amino-acid sequence have been used to isolate complementary DNA recombinants that correspond to the coding sequence of A4-amyloid¹⁷ and β -amyloid¹⁸⁻²⁰. The amyloid protein gene has been localized to chromosome 21 (refs 17-20) in the region of q11-q22^{19,20}. It has been suggested that the amyloid locus is segregating close to the Alzheimer's disease locus in the 21q21 region^{9,19}. Furthermore a duplication of the chromosomal region around the amyloid protein gene has been reported in sporadic Alzheimer's cases²¹. These observations have been assimilated into a single theory that predisposition to Alzheimer's disease is due to genetically determined over-expression of the β -amyloid protein, or expression of an abnormal variant of this protein^{18,19,21}.

Table 1 Lod scores for linkage

Histopathologically confirmed, early onset pedigrees	Recombinant fraction (θ)						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
Family AD/A	$-\infty$	-1.59	-0.73	-0.36	-0.08	-0.01	-0.02
AD/B	-0.63	-0.61	-0.55	-0.48	-0.34	-0.17	-0.03
Clinically diagnosed, late onset pedigrees*							
1	-1.15	-1.01	-0.69	-0.47	-0.22	-0.09	-0.02
2	-0.31	-0.31	-0.30	-0.28	-0.19	-0.09	-0.02
3	-0.22	-0.21	-0.19	-0.16	-0.09	-0.04	-0.01
4	+0.38	+0.37	+0.33	+0.28	+0.18	+0.09	+0.02
5	-0.33	-0.31	-0.24	-0.18	-0.09	-0.04	-0.01
Total (7 families)	$-\infty$	-3.67	-2.37	-1.65	-0.83	-0.35	-0.09

Values shown are lod scores for linkage between the A4-amyloid locus and Alzheimer's disease locus in seven informative families (two histologically confirmed). The exclusion limit is given by $z < -2$, $\theta = 0.07$.

The pedigrees used in this analysis were those of the two early-onset Alzheimer families illustrated in Fig. 1 (AD/A and AD/B) and five clinically diagnosed, late-onset pedigrees²⁴. In the early-onset pedigrees we used a gene frequency for the familial Alzheimer gene of 0.0001 (ref. 9) and assumed full penetrance of the disease. The late-onset pedigrees consisted of pedigree 1 (see Fig. 2, top) and four other informative sibships. These sibships contained 16 affected sibs with 21 unaffected sibs. The mean age of onset in all these clinically diagnosed families was between 55 and 70 years. An age of onset curve was constructed for each family to determine the risk for apparently unaffected family members. The gene frequency for an allele leading to familial Alzheimer's disease at an age of onset of ~ 65 was set at 0.01 (ref. 3). Calculation of the lod scores using these parameters for gene frequency and using an age-of-onset curve to determine the risk to all unaffected family members does not give an obligate recombinant in pedigree 1 because of the finite chances that the father of the sibship was an undiagnosed carrier of the familial Alzheimer trait or the mother was homozygous for the disease trait. All cases of Alzheimer's disease in the families were assumed to be of genetic rather than sporadic origin. These assumptions are justified because the clinical features of the disease within each family were homogeneous; however, recalculation of the data assuming less than full penetrance or assuming a low possibility (1%) that any individual case in a family is a sporadic case did not appreciably alter the exclusion limits (data not shown). Linkage was calculated using the MLINK program²⁵.

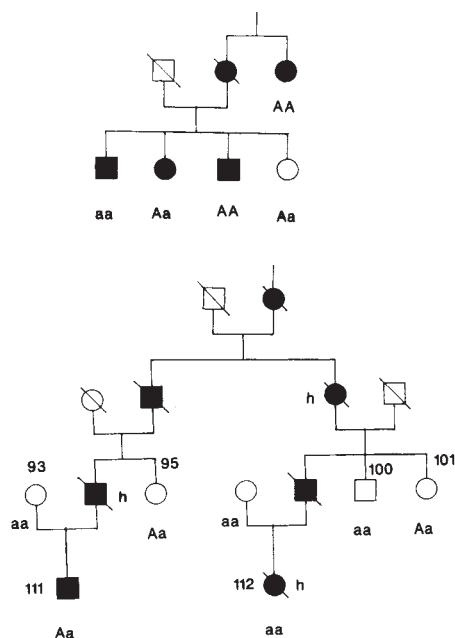


Fig. 2 Details of the haplotypes of pedigrees showing recombinations between Alzheimer's disease and the amyloid locus: h, histological confirmation at autopsy. The upper pedigree was diagnosed using the *American Psychiatric Association's Diagnostic and Statistics Manual*, 3rd edn criteria²⁴. The lower pedigree is a simplified section of the pedigree AD/A (Fig. 1). Symbols as in Fig. 1. Patient 111 is 33 years old and has been clinically diagnosed.

Methods. The *Bgl*II polymorphism used to trace the inheritance in pedigree analysis was detected using the 150-base pair (bp) *Sau*3A1 fragment of the amyloid precursor cDNA spanning the coding region for the A4 polypeptide (base pairs 1,770–1,920, ref. 17). This fragment detects two allelic *Bgl*II fragments of 9.6 kb (A) and 6.9 kb (a). The allele frequencies are 0.36 and 0.64 as determined in 76 unrelated Caucasians (probe information content value 0.35); the alleles in this population are in Hardy-Weinberg equilibrium. Codominant inheritance has been demonstrated in three extended families. The *Bgl*II RFLP is obscured by co-migrating constant bands if larger fragments of the cDNA clone are used as a probe. No polymorphisms were found using this 150-bp probe with *Acc*I, *Asp*700, *Ava*I, *Ava*II, *Bam*HI, *Ban*I, *Ban*II, *Bcl*I, *Bgl*I, *Bst*NI, *Bst*XI, *Cl*aI, *Dra*I, *Eco*RV, *Hind*II, *Hind*III, *Hin*fI, *Msp*I, *Nci*I, *Pst*I, *Pvu*II, *Rsa*I, *Sac*I, *Sau*96T, *Stu*I, *Taq*I and *Xba*I using a panel of six unrelated Caucasians. In 38 chromosomes, the polymorphism reported here appears to be in linkage equilibrium with the polymorphism detected by *Eco*RI reported previously^{18,19}.

We have identified a common restriction fragment length polymorphism (RFLP) of the A4-amyloid gene, and observed its segregation in two families with early onset (at <35 years of age, Fig. 1), and five families with late onset (~65 years of age) Alzheimer's dementia. These families show autosomal dominant transmission of Alzheimer's disease (Figs 1 and 2; see Fig. 2 legend for details of the polymorphism). Our linkage analysis (Table 1) shows that the gene for amyloid protein is not closely linked to the gene for familial Alzheimer's disease and in two families crossovers between the amyloid locus and the Alzheimer's locus are observed (Figs 2 and 3). Furthermore, we did not detect amyloid gene duplication on Southern blots in any of our cases²¹. The recombination between the familial Alzheimer's disease gene and the amyloid gene demonstrates that these loci are genetically distinct unless the recombination events occurred within the amyloid locus. The linkage data suggest that the amyloid locus is further away from the Alzheimer's locus than the anonymous probes to which linkage has been reported^{9,19}. Therefore, we studied the cosegregation of the amyloid polymorphism and a *Bgl*II polymorphism iden-

AD/A

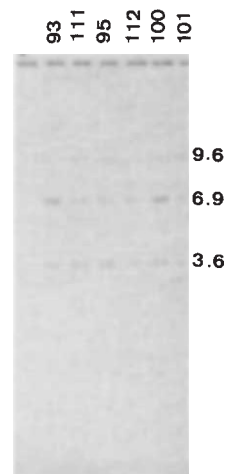


Fig. 3 Southern blot of members of pedigree AD/A (Fig. 2), showing the polymorphic bands at 9.6 kb (A) and 6.9 kb (a) and the constant band at 3.6 kb. Pedigree numbers are as in Fig. 2.

tified by the gene for superoxide dismutase (SOD); a marker localized at 21q22.1 (ref. 22). Preliminary data suggest that the amyloid protein gene may be linked to the SOD gene. (Maximum lod score, +1.92 at a recombination fraction of 0.07.) Such close proximity between the SOD gene and the amyloid gene has been recently reported in the homologous mouse chromosome using hybrid cell lines²³.

In conclusion our linkage data indicate that a mutation in the amyloid protein gene is not the primary defect causing familial Alzheimer's disease in all cases and suggest that the amyloid protein gene is closer to the 21q22 Down's syndrome phenotype region than has previously been reported¹⁹. Currently more Alzheimer's families are being investigated with both the amyloid and SOD polymorphisms to confirm these preliminary findings.

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The genetic defect in familial Alzheimer's disease is not tightly linked to the amyloid β -protein gene

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Amyloid β -protein (AP) is a peptide of relative molecular mass (M_r) 42,000 found in the senile plaques, cerebrovascular amyloid deposits, and neurofibrillary tangles of patients with Alzheimer's disease and Down's syndrome (trisomy 21)¹⁻⁴. Recent molecular genetic evidence has indicated that AP is encoded as part of a larger protein by a gene on chromosome 21 (refs 5-7). The defect in the inherited autosomal dominant form of Alzheimer's disease, familial Alzheimer's disease (FAD), has been mapped to the same approximate region of chromosome 21 by genetic linkage to anonymous DNA markers⁸, raising the possibility that this gene product, which could be important in the pathogenesis of Alzheimer's disease, is also the site of the inherited defect in FAD (ref. 5). We have determined the pattern of segregation of the AP gene in FAD pedigrees using restriction fragment length polymorphisms. The detection of several recombination events with FAD suggests that the AP gene is not the site of the inherited defect underlying this disorder.

If the AP gene were the site of the mutation causing FAD, extremely tight linkage between the two loci would be expected, with a virtually complete correlation of inheritance of a particular allele with the disease phenotype for any given FAD pedigree. We have previously reported a restriction fragment length polymorphism (RFLP) detected by the AP 3' complementary DNA clone FB63 which can be used to distinguish alleles at this locus in some individuals⁵. This RFLP displays allelic *EcoRI* fragments of 8.7 kilobases (kb) (allele 1) and 8.3 kb (allele 2). With frequencies of 0.94 and 0.06, respectively, for the two alleles, heterozygotes are uncommon. Consequently, the RFLP is of limited use as it is not informative in most pedigrees.

To increase the information from the AP gene as a polymorphic marker by identifying additional RFLPs, we have obtained a longer cDNA clone for the AP precursor by using the 3' cDNA FB63 as a probe to screen a λ gt10 cDNA library, constructed using messenger RNA from the promyelocytic leukaemia cell line HL60. Of the clones screened, ~0.2% hybridized positively to FB63 and ten were chosen for analysis. A 2.80-kb cDNA, HL12, consisted of three *EcoRI* fragments: a 1.05-kb piece analogous to FB63, flanked by a 1.50-kb fragment on the 5' side, and a 0.22-kb piece on the 3' side. Restriction map and partial sequence comparison of HL12 to the full-length AP cDNA clone reported by Kang *et al.*⁶ revealed that the clone represents all but the most 5' 430 base pair (bp) of the AP coding sequence. The 1.50-kb fragment was subcloned into the plasmid vector pUC18 for use as a probe and was designated HL124.

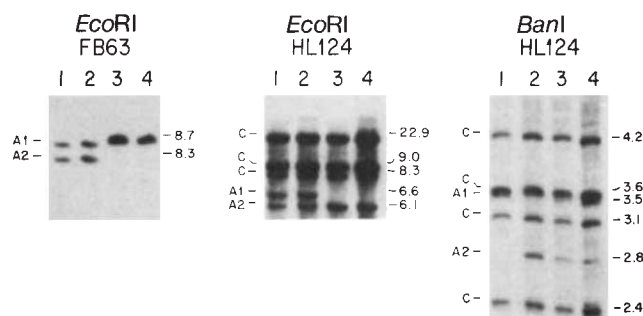


Fig. 1 RFLPs at the AP Locus. *EcoRI* RFLPs detected by FB63 and HL124, and a *BamI* RFLP detected by HL124 were monitored by Southern blot hybridization using the appropriate ³²P-labelled probe as previously described^{5,8}. Lanes 1, 3 and 4 contain genomic DNA digests from normal individuals; lane 2 in each represents DNA from a different FAD patient. Allelic fragments are designated as A1, allele 1, and A2, allele 2. 'C', Invariant fragments. A 2.9-kb constant *EcoRI* fragment detected by FB63⁵ was not detectable under the gel conditions used to increase the resolution of the allelic fragments. The enzymes for which no RFLP was seen with HL124 are: *BamHI*, *BglII*, *HindIII*, *MspI*, *PstI*, *SacI* and *TaqI*.

When hybridized to 10 different restriction enzyme digests of genomic DNAs from five unrelated individuals, HL124 revealed two RFLPs (Fig. 1): a second *EcoRI* RFLP independent of that detected by FB63, with allelic fragments of 6.6 kb and 6.1 kb, having frequencies of 0.38 and 0.62 ($n = 70$), respectively; and a *BamI* RFLP with allelic fragments of 3.5 kb and 2.8 kb, having frequencies of 0.82 and 0.18 ($N = 54$), respectively. The mendelian transmission of each RFLP was monitored by typing members of the Venezuela reference pedigree, a large collection of interrelated sibships used to construct a genetic linkage map of chromosome 21.

To determine whether the AP gene and the defect in FAD show complete cosegregation, inheritance of both *EcoRI* RFLPs and the *BamI* RFLP was traced in four large FAD pedigrees: FAD1, a large Canadian kindred of British descent; FAD2, a pedigree of German origin; FAD3, an American family of Russian ancestry; and FAD4, an extended pedigree with members in Italy, France and the United States. These kindreds, which display autosomal dominant transmission of the AD phenotype, were used previously in the recent discovery of genetic linkage between the FAD locus and DNA markers on chromosome 21⁸.

Linkage of the AP gene with FAD was assessed by computer analysis of the family data (using an age of onset correction^{8,9}) with MLINK from the LINKAGE package of programs¹⁰⁻¹². MLINK calculates the likelihood of linkage at various recombination fractions (θ) relative to the likelihood of non-linkage ($\theta = 0.50$). The $\log_{10}$ of each likelihood ratio or lod score (z) is shown in Table 1. A lod score < -2 is considered to exclude linkage to the disease locus; a lod score $> +3$ is considered proof of linkage.

The combined linkage data from all four FAD pedigrees yields a lod score of $-\infty$ at a recombination fraction $\theta = 0.0$ due to apparent crossovers between the AP locus and the FAD defect in every family. The presence of the FAD defect was excluded ($z < -2$) from a region of 8% recombination on either side of the AP gene. A total of six putative crossovers between the AP gene and FAD was observed with two recombination events in each of FAD3 and FAD4, and single crossovers in the other two pedigrees. Only one of the crossovers (in FAD4) was detected with the infrequent *EcoRI* RFLP of the FB63 probe, representing the 3' end of the coding sequence. All other crossovers were revealed by analysis of the two HL124 RFLPs, which are necessarily closer to the 5' end of the locus. As the age of onset in FAD is variable, even within the same pedigree, we have not counted as crossovers additional putative recombination events

Table 1 Lod scores for linkage of the AP protein gene to FAD

Pedigree	Recombination fraction (θ)						
	0.00	0.01	0.05	0.10	0.20	0.30	0.40
FAD1	$-\infty$	-2.00	-0.78	-0.39	-0.15	-0.07	-0.04
FAD2	$-\infty$	-1.92	-0.71	-0.26	0.05	0.05	0.10
FAD3	$-\infty$	-3.83	-2.28	-1.58	-0.91	-0.53	-0.38
FAD4	$-\infty$	-0.86	0.40	0.80	0.87	0.60	0.41
Total	$-\infty$	-8.61	-3.37	-1.43	-0.14	0.10	0.09

Exclusion ($z < -2$), $\theta < 0.08$.

Lod scores were calculated using MLINK of the LINKAGE package using an age of onset correction^{8,9}. A gene frequency of 0.0001 was assumed for the FAD defect. A family-specific curve was used for FAD4 and a general curve was used for the other families as described in the text⁸. Haplotype frequencies for the AP locus were estimated from those observed in 22 unrelated normal individuals where coupling phase of the individual RFLP alleles could be unequivocally determined. Considering alleles for the RFLPs (1 or 2 for each) in the order *Eco*RI (FB63), *Eco*RI (HL124) and *Ban*I (HL124), the haplotype frequencies used were as follows: '111', 0.36; '121', 0.40; '122', 0.14; '211', 0.06; and all others, 0.01 each.

in asymptomatic individuals, though these also contribute to the computer likelihood calculations.

The prominent deposition of AP in the brains of Alzheimer's disease patients suggests that the protein could be important in the pathogenesis of the disorder. The localization of the AP gene to the same general chromosomal region as the FAD gene has led to speculation that a mutation at the AP locus might underlie this inherited form of AD. The evidence presented here seems to exclude the defect from the immediate genetic vicinity of the AP gene. It is conceivable that, like the Duchenne muscular dystrophy gene¹³, the AP gene spans a very large genetic distance with the FAD mutation occurring at the extreme 5' end, but the generally low frequency of recombination in this region of chromosome 21¹⁴ argues against this hypothesis. Preliminary evidence places the AP gene about 7.5 centimorgans distal to the *D21S1/D21S11* locus, excluding FAD from the region between these two loci and suggesting a more proximal location for the defect. If the average recombination rate for the human genome is applied to this area, the formally excluded segment would include $\sim 8 \times 10^6$ bp on either side of the AP locus.

The neuropathological features typical of AD are also observed in older individuals with Down's syndrome, suggesting that overexpression of a gene or set of genes on chromosome 21 can produce the disease phenotype. We have previously demonstrated overexpression of the AP gene in Down's syndrome⁵, but it is not known whether the increased amount of the AP protein leads directly to formation of amyloid deposits. The discovery that the defect causing FAD is unlikely to be located in or near the AP gene suggests that the disorder is either caused by altered expression of a second independent gene on chromosome 21 (probably overexpressed along with the AP gene in Down's syndrome), or by a long range effect of the defect on the expression of the AP gene. The latter could arise from a structural abnormality in chromosome 21 causing overexpression of AP either by promoting mitotic nondisjunction leading to somatic cells trisomic for this autosome, or by duplication of a large region of the chromosome containing the AP gene. Increased dosage of the AP gene has been reported in sporadic Alzheimer's disease¹⁵, but is not observed in affected members of our FAD kindreds, which eliminates this mechanism as the primary cause of the inherited form of the disorder¹⁶. If the FAD defect lies in a gene encoding some other protein responsible for triggering the FAD phenotype, it should eventually be possible to isolate this gene from its location. If the mutation underlying FAD directly alters the expression of the AP gene due to a distant *cis*-acting element, however, it could prove quite difficult to identify the culprit DNA sequence.

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Transient expression of IL-2 receptor precedes the differentiation of immature thymocytes

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The growth of mature T lymphocytes after activation by antigen is regulated by the binding and endocytosis of interleukin-2 (IL-2)^{1,2}. In the thymus, $\sim 50\%$ of adult thymocytes that carry neither the CD4 nor the CD8 antigen and day 14-15 fetal CD4⁺CD8⁻ thymocytes express receptors for IL-2 (IL-2R)³⁻⁵. The CD4⁺CD8⁻ (double-negative) subpopulation of thymocytes contains the precursors of cells that can differentiate along an unknown pathway into thymocytes bearing either CD8 or CD4, with the characteristics of mature T lymphocytes^{6,7}. The basis for IL-2R expression by double-negative thymocytes is unclear as they appear to lack a functional T-cell receptor/CD3 complex through which activation of peripheral T cells is mediated⁸. The argument for a role for IL-2 in thymocyte differentiation has also been complicated by conflicting reports on the inability⁹⁻¹² or capability^{4,13} of double-negative thymocytes to respond to IL-2 *in vitro*. At present, both the nature of the stimuli within the thymic micro-environment which induce IL-2R expression and its relevance to thymocyte differentiation are not known. We show here that the IL-2R-bearing subset has a greater potential to differentiate into phenotypically mature T lymphocytes than do IL-2R-negative thymocytes. In addition, progeny of IL-2R-negative donor cells transiently express IL-2R in the thymuses of adoptive hosts before generating CD8 and/or CD4-positive thymocytes. These results identify the IL-2R-positive cells as a more differentiated double-negative thymocyte subset on the pathway to mature T lymphocytes.

Adoptive intrathymic transfer of unfractionated adult CD4⁺CD8⁻ thymocytes into irradiated Thy-1 congenic recipients resulted in a wave of donor cell repopulation that peaked at

about day 12 in the host thymus and at ~3–4 weeks in host lymph nodes and spleen (Table 1). Early after cell transfer we recovered fewer donor-type cells than were injected, at day 5 only 68% of the cell numbers originally injected. One might expect the loss of viable cells to be due to non-specific causes during cell preparation and the injection procedure. It is also possible that the observed repopulation was effected by only a minor proportion of the cells transferred. By days 10–15 post-transfer, the Thy-1.1-bearing double-negative donor cells had expanded several-fold and constituted a major proportion of the thymocyte population in the adoptive host. Progeny of the transferred thymocytes underwent apparently normal intrathymic differentiation, as they acquired CD8 and CD4 expression typical of normal thymocytes (Fig. 1) and yielded, in peripheral lymph organs, CD8 and CD4 single-positive cells which were functionally competent in mixed lymphocyte culture (R.S., manuscript in preparation). Donor cells disappeared from adoptive host thymuses by day 21, but could be detected in the periphery for up to 34 (and in other experiments, 110) days post-transfer.

When fluorescence-activated cell sorted (FACS) IL-2R-positive or IL-2R-negative double-negative thymocytes were transferred intrathymically, significant differences in the pattern of reconstitution were apparent. Repopulation of adoptive host thymuses receiving IL-2R-positive double-negative cells peaked earlier and yielded greater numbers of donor-derived cells than did thymuses receiving equivalent numbers of IL-2R-negative cells (Table 2), indicating that IL-2R-bearing thymocytes were more efficient at thymic reconstitution than their receptor-negative counterparts. The recovery of Thy-1.1-bearing donor-derived cells on day 5 post-transfer was 200% and 8%, for mice receiving IL-2R-positive and negative cells, respectively (Table 2, Experiment 1). The small size of these yields may be due to nonspecific cell death but the result demonstrates the greater capacity of the IL-2R-positive thymocytes for recolonization. In addition to these quantitative differences, the transition of double-negative cells to CD8/CD4 double-positive thymocytes and the appearance of CD4 single-positive cells occurred earlier in thymuses reconstituted with IL-2R-positive cells than in those reconstituted with IL-2R-negative cells (days 5–10 and 10–15, respectively, Fig. 1). Particularly striking was the expression by day 5 of either CD8 or both CD8 and CD4 by the majority (86%) of the progeny of transferred IL-2R-positive donor cells. (Insufficient cell numbers after depletion of host cells using anti-Thy-1.2 antibody plus complement did not allow analysis of these markers in mice which received IL-2R-negative and total double-negative cells.) By day 10 in IL-2R-positive recipients double-negative cells constituted only 3% of the donor Thy-1.1 population, which had become CD8/CD4 double-positive (75%) and CD8 or CD4 single-positive (11% and 9%, respectively). In contrast, at day 10 the progeny of IL-2R-negative donor cells were still 30% double-negative and not until day 15 were all of the mature sub-populations apparent. This acceleration in the expression of CD8 and CD4, as well as the numbers of donor cell progeny found in host lymph nodes

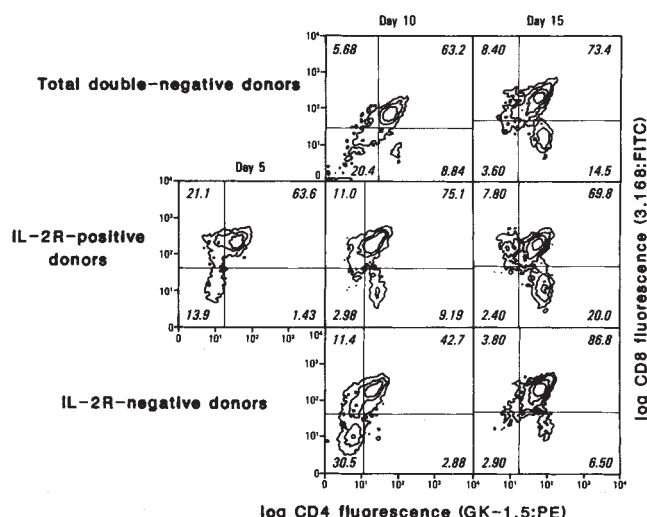


Fig. 1 Repopulation of adoptive host thymuses by the progeny of intrathymically transferred Thy-1-congenic donor thymocytes. Dual fluorescence analysis for CD8 and CD4 in adoptive host thymuses recolonized by total double-negative, IL-2R-positive and IL-2R-negative double-negative thymocytes is shown. Thymuses were depleted of host-derived cells as described in Table 2 and stained with 3.168 : FITC (anti-CD8) and GK-1.5 : PE (ref. 27; anti-CD4) and analysed on the Becton-Dickinson FACS IV. Dead cells were excluded by selective gating and the quadrants shown define immunofluorescence above that of the background (the quadrant defined by CD8⁻CD4⁻ cells). The numbers in the quadrants refer to the percentage of positively staining cells corrected for background fluorescence. Day 5 staining in total CD4⁺8⁻ and IL-2R-negative CD4⁺8⁻ thymuses was not attempted due to low cell yields.

at days 10 and 15 (Table 2), indicated a quicker course through differentiation and export from the thymus by the progeny of IL-2R-positive donors.

The acquisition of IL-2R expression by IL-2R-negative CD4⁺8⁻ donors was detected in adoptive host thymuses by day 5 post-transfer (Table 2). The progeny of FACS-sorted IL-2R-negative donor cells were 29% IL-2R-positive at day 5 and 7% positive at day 10. In contrast, the progeny of IL-2R-positive cells were 6% IL-2R-positive at day 5 and negative at day 10, indicating the loss of IL-2R expression. Both transferred populations showed a decrease in IL-2R expression that correlated with an increase in expression of CD8 and CD4 (see Fig. 1). The data indicate that IL-2R-positive donor cells lose IL-2R expression as they become CD8/CD4-positive (by day 5) whereas the progeny of IL-2R-negative double-negative donors only acquire CD8 and CD4 after the transient expression of IL-2R.

The proportion of cells in the thymus expressing IL-2R are only a minority of those cells which are blasts¹⁴. Similarly, our unpublished data indicate that both IL-2R-positive and negative

Table 1 Recolonization of the thymus and periphery by intrathymically transferred double-negative thymocytes

Organ	% Donor-derived cells (number × 10 ⁶) on day post-transfer					
	5	10	15	20	27	34
Thymus	80 (2.7)	63 (38)	38 (42)	9.6 (7.8)	0.5 (0.5)	0.9 (1.2)
Mesenteric lymph node	0	9.1 (0.1)	26 (0.2)	28 (2.0)	21 (3.1)	19 (1.9)
Spleen	0	0	2.5 (3.3)	9.5 (9.7)	9.8 (7.8)	6.5 (6.2)

For donor cells, double-negative thymocytes were prepared from 6-week-old B6.PL Thy-1^{cy} mice using two cycles of treatment with 3.168 (ref. 22; anti-CD8) and RL-172.4 (ref. 3; anti-CD4) antibodies plus guinea-pig complement. Analysis of donor cells indicated >95% double-negative cells and ~5% dull CD4-positive cells. Double-negative thymocytes (4 × 10⁶) were transferred intrathymically via the method of Goldschneider *et al.*²³ into 950 rad-irradiated, T-depleted syngeneic bone marrow-reconstituted C57Bl/6 mice. Donor-derived cells were detected on the indicated days post transfer using the Thy-1.1-specific monoclonal antibody 19E12 (ref. 24) and FITC-goat anti-mouse Fc on the FACS. Absolute numbers of donor-derived cells were calculated from these percentages and the total cell yield per organ.

Table 2 Thymus and lymph node recolonization by intrathymically transferred FACS-sorted IL-2R-positive or IL-2R-negative CD4⁺ 8⁺ thymocytes

Day post transfer	Specific immunofluorescence	% Fluorescence-positive cells (number $\times 10^6$) in adoptive hosts receiving					
		IL-2R-positive cells		IL-2R-negative cells		total CD4 ⁺ 8 ⁺ cells	
		total	Thy-1.2 depleted	total	Thy-1.2 depleted	total	Thy-1.2 depleted
Experiment 1							
Day 5	total Thy-1	61.3 (0.8)	†	60.1 (0.4)			
Thymus	Thy-1.1	33.7 (0.5)		3.1 (0.02)			
	IL-2R	3.6 (0.05)	6.2	7.5 (0.05)	29.8		
	total Thy-1	98.8 (77)	97.5	99.0 (84)	88.2		
Day 10	Thy-1.1	30.0 (24)	93.2	6.9 (5.9)	74.6		
Thymus	IL-2R	0.3 (0.2)	0.4	1.2 (1.0)	8.7		
Experiment 2							
Day 10	total Thy-1	95.4 (34)	96.4	96.1 (24)	81.1	94.5 (26)	94.1
Thymus	Thy-1.1	74.0 (26)	94.4	11.3 (2.8)	79.9	37.2 (10)	90.1
	IL-2R		0.0		7.1		1.6
	total Thy-1	71.9 (0.2)		53.8 (0.3)		49.3 (0.4)	
Day 10	Thy-1.1	16.6 (0.04)		5.7 (0.03)		4.4 (0.03)	
Day 15	total Thy-1	95.5 (60)	99.3	92.1 (74)	98.6	94.1 (78)	98.7
	Thy-1.1	46.1 (29)	99.2	37.3 (30)	99.0	20.0 (17)	98.4
	IL-2R		1.1		0.4		0.0
Day 15	total Thy-1	30.7 (0.8)	19.5	29.8 (1.2)	5.6	24.1 (0.8)	5.9
Lymph node	Thy-1.1	16.2 (0.5)	19.2	2.6 (0.1)	3.2	5.5 (0.2)	4.4

All % values are corrected for second-reagent background staining. Cell numbers were calculated from the immunofluorescence percentages obtained and the total cell yield per organ. Double-negative thymocyte donors were prepared as described in Table 1 and showed 0.8% dull CD8-specific and 0.09% dull CD4-specific immunofluorescence upon reanalysis in experiment 1 and 2.45% and 5.07%, respectively, in experiment 2. Sorting on a Becton-Dickinson FACStar yielded 98.2% IL-2R-positive and 99.5% IL-2R-negative cells in experiment 1, and 97.1% and 99.3%, respectively, in experiment 2 from starting double-negative populations that were ~50% IL-2R-positive. Total double-negative donors were an identical aliquot of cells stained for IL-2R but not sorted. Adoptive hosts received 2.5×10^5 (exp. 1) or 1×10^6 (exp. 2) FACS-sorted IL-2R-positive or IL-2R-negative double-negative or total double-negative donor cells. Cells recovered from organs on the indicated days were stained using Thy-1-specific (T24, ref. 25), Thy-1.1 allele-specific (19E12) and IL-2R-specific (PC-61, ref. 3) monoclonal antibodies and a FITC-conjugated goat anti-mouse Ig (rat Ig cross-reactive) second antibody. Thymus and lymph nodes were depleted of Thy-1.2-positive (host) cells using the 13-4 (ref. 26) monoclonal antibody and guinea pig complement. Re-analysis of Thy-1.2-specific immunofluorescence after treatment indicated <0.5% residual host-derived Thy-1.2 cells remaining.

† Absence of a value indicates insufficient cells to analyse.

CD4⁺ 8⁺ thymocyte subpopulations contain blast cells as discerned by forward light scatter on the Becton-Dickinson FACS IV. Because the IL-2R is not expressed on the CD8/CD4 double and single-positive blast subpopulations, IL-2 seems to be important only for the double-negative subset. Ligand-binding studies show IL-2R on double-negative thymocytes to be incapable of internalizing IL-2 (ref. 12), which may reflect either an inability to express or a previous expression and subsequent down-regulation of IL-2R α -chain protein^{15,16}. The former possibility is difficult to reconcile with the stimulation of IL-2R-negative CD4⁺ 8⁺ thymocytes to proliferate by PMA plus ionomycin^{10,11,17}. This combination of *in vitro* mitogenic signals can apparently induce the expression of high affinity IL-2R on double-negative thymocytes and allow IL-2-dependent growth¹². But the *in vivo* stimulatory signals are unclear, and if IL-2 is important in thymic differentiation our results imply that it may be involved in the expansion of the intrathymic pool of multi-potential double-negative T-cell precursors.

The experiments of Hyman *et al.*¹⁸ suggest that thymus-homing progenitor activity after intravenous transfer is a function of IL-2R-negative, Pgp-1-positive but not IL-2R-positive, Pgp-1-negative CD4⁺ 8⁺ thymocytes. The data presented here show IL-2R-positive cells to be fully capable of reconstituting adoptive host thymuses with phenotypically mature thymocyte populations when transferred intrathymically. Together, these two lines of evidence indicate that IL-2R-positive thymocytes are a more differentiated double-negative subset which have lost thymus-homing receptors.

The pathway by which double-negative thymocytes differentiate into CD8- and CD4-single-positive mature T-cell precursors remains obscure. The CD8/CD4 phenotype of the progeny of IL-2R-positive double-negative donors in day 5 adoptive host thymuses (Fig. 1) as well as our unpublished data on day 16-17 BALB/c fetal thymocytes suggest an initial double-negative to single CD8-positive transition *in vivo* with CD8/CD4 double-positive cells appearing immediately afterwards. This agrees with a report on the acquisition of CD8 before CD4 by double-negative thymocytes in sheep fetal ontogeny¹⁹, and with the appearance of CD8 and CD4 in murine fetal thymic organ

culture *in vitro*²⁰. The observation that single CD4-positive cells appear later suggests they have a slower maturation rate. We did not attempt to determine here if individual IL-2R-positive double-negative cells were committed to a particular lineage (CD8 or CD4 single-positive) or if they were multi-potential on a clonal level. As CD8 and CD4 proteins expressed by peripheral T lymphocytes may be important in antigen recognition²¹, CD8/CD4 lineage commitment by IL-2R-positive thymocytes might be coordinated with the expression of T-cell receptor protein.

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Importance of IL-2 receptors in intra-thymic generation of cells expressing T-cell receptors

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During development, lymphoid stem cells migrate into the thymic rudiment where they proliferate¹, rearrange their antigen receptor genes²⁻⁴ and become differentiated into functionally mature T cells⁵⁻⁷. At present, the regulation of these processes is poorly understood, although recent studies have shown that early fetal and adult immature thymocytes express receptors for the T-cell growth factor, interleukin-2 (IL-2)⁸⁻¹¹. We now present direct evidence that IL-2 receptors have a function in intra-thymic development by demonstrating that proliferation and the generation of cells expressing the T-cell antigen receptor ($\alpha\beta$ TCR), which is responsible for the recognition of antigens in the context of MHC^{12,13}, are inhibited when antibodies to IL-2 receptors are added to fetal thymus organ cultures. The inhibition is specific in that it does not affect pre-thymic stem cells and can be partially reversed by addition of exogenous recombinant IL-2.

Previous reports have shown that the monoclonal antibody PC.61, which recognizes IL-2 receptors, inhibits IL-2 binding⁸ and prevents the IL-2-dependent proliferation of mature T cells¹⁴. To investigate whether the binding of IL-2 to IL-2 receptors on precursor cells is important in stimulating cell proliferation within the embryonic thymus, PC.61 was added to organ cultures of fetal thymus rudiments explanted at d14 of gestation. At this stage the majority of thymocytes have an immature (CD4⁺ CD8⁻) phenotype and carry the IL-2 receptor⁸. As shown in Fig. 1, addition of PC.61 at a final concentration of 10 $\mu\text{g ml}^{-1}$ produces a dramatic reduction in cell yield compared with control cultures of similar duration.

In contrast, organ cultures of fetal liver treated with PC.61 retain their ability to colonize alymphoid embryonic thymus lobes when associated with them in a transfilter culture system using combinations of fetal liver and thymus differing at the Thy-1 locus as described previously¹⁵. Thus 12/12 thymus lobes associated with PC.61-treated fetal liver fragments were successfully recolonized and generated numbers of cells comparable to control cultures recolonized by untreated fetal liver, with the majority of cells (>80%) expressing Thy-1 of fetal liver donor type. This observation is in agreement with immunolabelling studies suggesting that IL-2 receptors are not expressed before entry of stem cells into the thymus¹⁰. Our results also indicate that the inhibitory effect noted in thymus organ cultures is not due to a general toxic effect of PC.61 on embryonic cells *in vitro*.

To provide further evidence that the inhibitory effect of PC.61 antibody is due to blocking the interaction of IL-2 with IL-2 receptor, exogenous recombinant IL-2 was added to thymus organ cultures treated with PC.61 in an attempt to overcome the inhibitory effect of the latter. Previous studies have shown that addition of exogenous IL-2 can reverse the inhibitory effect of PC.61 on the proliferation of T-cell lines¹⁴ presumably because a greater concentration of IL-2 molecules can compete more effectively with PC.61 for binding to IL-2 receptors. At a PC.61 concentration of 10 $\mu\text{g ml}^{-1}$ only marginal rescue of intrathymic proliferation by IL-2 was observed (data not shown). But at a PC.61 concentration of 5 $\mu\text{g ml}^{-1}$ rescue from inhibition was obtained when IL-2 (50 U ml^{-1}) was present (Fig. 2). Thus it is likely that the IL-2 growth factor system is used during precursor cell proliferation within the thymus. An alternative possibility, namely that the presence of antibody to IL-2 receptors results in opsonization of cells by macrophages is unlikely

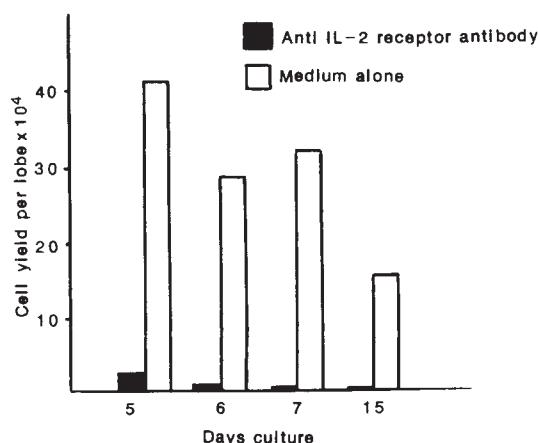


Fig. 1 The effect of anti-IL-2 receptor antibody on 14-day embryo thymus organ cultures. Fetal thymic rudiments were obtained from timed matings of CBA mice with the day of detection of a vaginal plug designated day 0. Lobes isolated at day 14 of gestation were organ-cultured on the surface of nucleopore filters resting on gelatin foam sponges as described previously¹⁵. Monoclonal antibody PC.61 purified by protein-A sepharose chromatography (a kind gift from Dr H. R. MacDonald) was added to organ cultures at a concentration of 10 $\mu\text{g ml}^{-1}$. Control cultures were established under the same conditions but without PC.61. Experimental and control cultures of the same duration (5–15 days) were harvested, mechanically disaggregated and the resultant cell suspension counted to determine cell yield per lobe. All counts were based on a minimum of 5 lobes per experimental or control group. The results demonstrate a marked reduction of cell yield in PC.61 treated as compared to control cultures suggesting that blocking the IL-2 receptor inhibits T-cell precursor proliferation within the thymus.

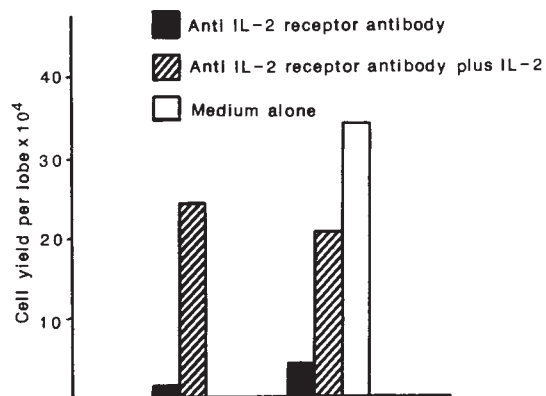


Fig. 2 Recovery of yield in antibody-treated cultures by addition of IL-2. Organ cultures were carried out as described in the legend to Fig. 1 except that PC.61 was added at a final concentration of 5 $\mu\text{g ml}^{-1}$ and some cultures received recombinant IL-2 (Amersham) at a final concentration of 50 U ml^{-1} . After 8 days culture lobes were harvested, disaggregated and cell counts carried out to determine cell yield per lobe. Each group contained a minimum of four lobes. Note that PC.61 at 5 $\mu\text{g ml}^{-1}$ causes a marked reduction in cell yield compared to control cultures but this can be partially reversed when recombinant IL-2 is added. To demonstrate that these effects included the cell lineage undergoing T-cell receptor expression, cells in the right-hand panel were examined for T-cell receptor β -chain expression—Table 1.

because we have found no evidence in electron micrographs (data not shown) of internalization of lymphocytes within the macrophages present in thymic organ cultures. Furthermore, few macrophages are found in the early thymus when IL-2 receptor expression is at a peak¹⁶.

It is also important to note that the recovery of cell numbers after addition of exogenous IL-2 results in proportions of cells

Table 1 Prevention of the inhibitory effect of anti-IL-2 receptor antibody by the addition of recombinant IL-2

Treatment	Total cells counted	% Cytoplasmic labelling	% Surface labelling	Total % positive
PC.61 alone (5 μ g ml ⁻¹)	318	2.20	2.25	4.45
PC.61 + 50 U ml ⁻¹ IL-2	413	4.60	7.26	11.86
Medium + 50 U ml ⁻¹ IL-2	369	6.78	8.40	15.18
Medium alone	433	4.62	7.39	12.01

Surface and cytoplasmic labelling for the β -chain of the T-cell antigen receptor was carried out on the cells from the right-hand panel in Fig. 2 using monoclonal antibody F-23.1 (a kind gift from Dr M. Bevan). This antibody recognizes a V_{β} gene family detectable on ~25% of peripheral T cells and 10-12% of adult thymocytes¹⁷. Briefly, cell suspensions were labelled sequentially with F23.1, anti-mouse immunoglobulin biotin and avidin-rhodamine. Labelled suspensions were then cytospun onto slides, fixed in cold methanol containing 5% acetic acid and incubated sequentially with F23.1, anti-mouse immunoglobulin biotin and streptavidin-fluorescein as described previously²¹. Slides were mounted in anti-fade compound (DABCO) and examined in a Zeiss epi-illumination microscope for surface (both rhodamine and fluorescein) and/or cytoplasmic (fluorescein only) labelling. The reduction in both surface and cytoplasmic F-23.1⁺ cells in PC.61 treated cultures indicates that the inhibitory effects of PC.61 treatment includes cells from the lineage destined to undergo T-cell receptor β -chain gene rearrangement and expression. The specificity of this effect is shown by the restoration of both surface and cytoplasmic F-23.1⁺ cells to control proportions when recombinant IL-2 is added.

Table 2 Detection of IL-2 receptors on embryonic thymocytes expressing cytoplasmic TCR β -chain but not on thymocytes expressing surface TCR β -chain

		% F23.1	% F23.1 cells IL-2R	Total number cells scored
16 days gestation				
Staining for surface β -chains	(exp 1)	0.55	0	362
	(exp 2)	0.32	0	311
Staining for surface and cytoplasmic β -chains	(exp 1)	3.31	18.75	511
	(exp 2)	1.97	29.17	1,219
18 days gestation				
Staining for surface β -chains	(exp 1)	5.22	0	805
	(exp 2)	2.88	0	763
Staining for surface and cytoplasmic β -chains	(exp 1)	8.22	9.49	1,156
	(exp 2)	6.21	13.61	709

IL-2 receptors were detected by incubating thymocyte suspensions with the rat monoclonal antibody PC.61 followed by a goat anti-rat immunoglobulin rhodamine conjugate (at 16 days gestation 53% of cells carried the IL-2 receptor, whereas at 18 days gestation 8% of cells carried the IL-2 receptor). For surface β -chain detection, the cells were incubated in the mouse monoclonal antibody F23.1 which detects products of a V_{β} gene family present on about 1 in 3 T cells¹⁷, followed by goat anti-mouse immunoglobulin biotin and finally streptavidin-fluorescein. For surface and cytoplasmic β -chain detection, suspensions of thymocytes stained for IL-2 receptors as above were cytospun onto slides, fixed in acid-alcohol and washed in phosphate-buffered saline. The cells were then treated in sequence with F23.1 antibody, anti-mouse immunoglobulin biotin and streptavidin fluorescein. At 16 days gestation, most F23.1⁺ cells have cytoplasmic but not surface β -chains—hence, the proportion of cells expressing IL-2 receptors in cytopins is a reasonable estimate of IL-2 receptor-bearing cytoplasmic cells. But at 18 days gestation, surface β -chain-bearing cells are present in greater numbers, hence the percentage of F23.1-bearing cells in cytopins that also carry the IL-2 receptor gives an underestimate of the proportion of cytoplasmic β -chain-bearing cells that also carry the IL-2 receptor.

labelled with the F23.1 antibody (which recognizes a product of a V_{β} family of the $\alpha\beta$ TCR; see ref. 17) approaching those found in control cultures (Table 1). Recent studies have shown that immature thymocytes stimulated to proliferate outside the thymic microenvironment in the presence of IL-2 do not express the $\alpha\beta$ TCR but may express the γ TCR¹⁸⁻²⁰. Thus it might be argued that rescue by IL-2 within the thymus involves preferential expansion of this or some other non-representative subpopulation. But our studies suggest that the IL-2 system is used within the $\alpha\beta$ T-cell lineage which makes up the majority of thymus lymphocytes, and this is further confirmed by the fact that IL-2 receptors can be detected on thymocytes expressing cytoplasmic TCR β -chains (Table 2), although these disappear by the time surface TCR expression is achieved. This pattern of IL-2 receptor expression correlates with our earlier finding that thymocytes which have detectable β chain in the cytoplasm are in cell cycle whereas newly-formed thymocytes expressing surface β -chains have left cell cycle²¹.

A functional role for IL-2 receptors in early T-cell development also implies that IL-2 is available within the thymus at an early stage. In this context Ceredig¹⁴ has shown that 14d thymocytes stimulated with PMA and ionomycin can produce IL-2. How IL-2 production is triggered within the thymus and whether this is a function of all immature thymocytes or of a particular

subset remains to be established. Similarly, the signals inducing IL-2 receptor expression following entry of precursors into the thymus are also unknown. It has been proposed that CD2 molecules on precursor cells interact with a natural ligand on thymic stromal cells and so activate the IL-2 system²². But we have detected membrane IL-2 receptors before CD2 transcripts in murine thymus ontogeny (M. Owen, E. J. J. and J.J.T.O., unpublished observations), arguing against this proposal.

Our results provide direct evidence that the IL-2 system is used during proliferation of T-cell precursors within the embryonic thymus. This does not, however, exclude a role for other growth factors or the possibility that not all thymocyte proliferation is IL-2 dependent²³. In addition, recent studies have shown that freshly isolated IL-2 receptor-bearing thymocytes do not endocytose IL-2 or proliferate in its presence without additional stimuli²⁴. These observations, together with the finding that precursor cells stimulated to proliferate outside the thymic microenvironment also fail to undergo full TCR β -chain gene rearrangement¹⁸⁻²⁰, indicate that additional signals available within the thymus are required for full T-cell maturation.

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Antibacterial agents specifically inhibiting lipopolysaccharide synthesis

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The spread of antibiotic resistance in Gram-negative bacteria has sustained a continuing search for new agents with antibacterial activity against this important class of bacterial pathogen. Because the biosynthesis of lipopolysaccharide (LPS) is unique to Gram-negative bacteria and required by them for growth and virulence, attempts have been made to discover or design antibacterial agents acting at this site; however, no such agents have so far been developed¹. We now present definitive experimental data documenting design of the first member of the class of antibacterial compounds which specifically inhibit LPS synthesis. The target enzyme is 3-deoxy-D-manno-octulosonate cytidylyltransferase (CMP-KDO synthetase), a cytoplasmic enzyme which activates 3-deoxy-D-manno-octulosonate (KDO) for incorporation into LPS. A specific inhibitor of CMP-KDO synthetase, α -C-(1,5-anhydro-7-amino-2,7-dideoxy-D-manno-heptopyranosyl)-carboxylate was designed using results of our studies of the purified enzyme. LPS synthesis ceased and lipid A precursor accumulated, causing growth stasis and perturbation of outer membrane structure and function, following delivery of the inhibitor to the intracellular target by a peptide carrier. Antibacterial action required an intact oligopeptide permease system and specific intracellular aminopeptidase activity to release inhibitor from the peptide prodrug.

The enzyme CMP-KDO synthetase is unique to Gram-negative bacteria and activates KDO for incorporation into LPS:



Laboratory strains of *Salmonella typhimurium* containing genes *kdsA* or *kdsB*, coding for temperature-sensitive KDO-8-phosphate synthetase or CMP-KDO synthetase respectively, stop LPS synthesis and accumulate lipid A precursor at the restrictive temperature (42 °C), leading to bacteriostasis^{2,3} (M. J. Osborn, unpublished data). Virulent strains (LD₅₀ = 100 colony forming units or CFU) become avirulent (LD₅₀ > 10⁷ CFU) for mice when the temperature-sensitive allele of *kdsB* is substituted for

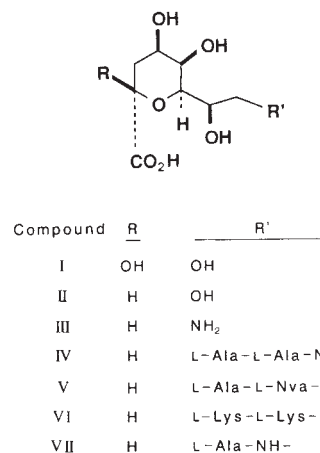


Fig. 1 Structures of KDO, CMP-KDO synthetase inhibitors and peptide prodrugs. Nva, norvaline.

the normal allele, because lipid A precursor accumulates in the outer membrane at 38 °C (mouse body temperature) rendering bacteria susceptible to clearance by cells of the reticuloendothelial system⁴. These bacteria also become susceptible to complement mediated killing following subsequent incubation at 42 °C, because lipid A precursor reaches the outer membrane and causes structural perturbations which allow stable insertion of the complement membrane attack complex⁵. It was therefore predicted that a suitable chemical inhibitor of CMP-KDO synthetase would have similar effects on all Gram-negative bacteria which incorporate KDO into LPS by this mechanism.

We have characterized the structure and substrate specificity of CMP-KDO synthetase cloned from *Escherichia coli* K12 (refs 6-9). The β -pyranose form of KDO(I), the least abundant of four solution tautomers, was the actual substrate used in forming CMP-KDO (ref. 9). This led to synthesis of α -C-(1,5-anhydro-2,7-dideoxy-D-manno-heptopyranosyl)-carboxylate (II), a 2-deoxy derivative locked in the β -pyranose configuration, which competitively inhibits CMP-KDO synthetase (K_i = 12 μ M). Further studies led to synthesis of α -C-(1,5-anhydro-7-amino-2,7-dideoxy-D-manno-heptopyranosyl)-carboxylate (III) (see Fig. 1) which is a more effective competitive inhibitor of CMP-KDO synthetase (K_i = 4 μ M). Because compound III did not enter bacteria, it was attached to the carboxyl terminus of dipeptides in amide linkage (Fig. 1) to effect transport by peptide permeases. This approach is an extension of original studies demonstrating the use of peptide carriers for transport of non-permeating, inhibitory agents^{10,11}.

LPS synthesis ceased, lipid A precursor accumulated, and bacteriostasis resulted (Fig. 2) following treatment of virulent *S. typhimurium* with compound IV, a peptide prodrug of III (see Fig. 1); the minimum inhibiting concentration (MIC) for inhibition of growth was 6 μ g ml⁻¹. The major drug-induced lipid A precursor species were identical to those that accumulated after thermal inhibition of bacterial strains containing temperature-sensitive KDO-8-phosphate^{12,13} or CMP-KDO synthetase enzymes (Fig. 2c), and the basic structure was confirmed (R.C.G. *et al.*, in preparation) as O-(2-amino-2-deoxy- β -D-glucopyranosyl)-(1-6)-2-amino-2-deoxy- α -D-glucose, acylated at positions 2, 3, 2' and 3' with β -hydroxymyristoyl moieties and bearing phosphate groups at positions 1 and 4', by compositional analysis and FAB mass spectroscopy (see Fig. 2 legend for a description of subspecies). Lipid A precursor molecules of similar structure accumulate when diverse species of Gram-negative bacteria (*Serratia*, *Providencia*, *Enterobacter*, *Citrobacter*, *Escherichia* and *Pseudomonas*) are treated with compound IV, or related antibacterial agents (R.C.G. *et al.*, in preparation); MIC values for inhibition of growth ranged from 5 to 100 μ g ml⁻¹ depending on the species and agent tested.

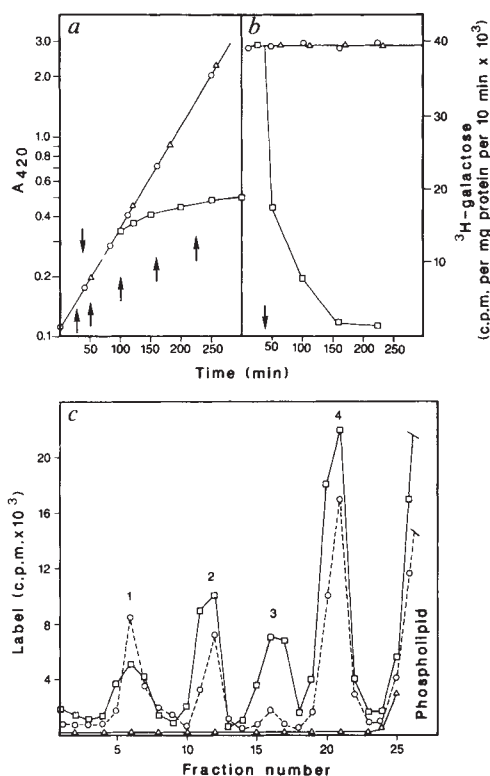


Fig. 2 Inhibition of growth and LPS synthesis by compound IV. *a, b*, A *galE* mutant of *S. typhimurium* LT2 was grown in MOPS defined medium¹⁶ containing 0.4% (v/v) glycerol and 100 μ M galactose. Drug, 100 μ g ml⁻¹ ($\square$, compound IV; or Δ , compound III) or no drug ($\circ$, control) was added, at times shown by downward arrows. Growth (as A₄₂₀) was measured in *a*; LPS synthesis (as ³H-galactose incorporation, triplicate determinations) in *b*. Upward arrows in *a* show the time of sampling for ³H-galactose incorporation. Note that ³H-galactose is only incorporated into LPS in *galE* mutants. *c*, Induction of lipid A precursor accumulation by compound IV. $\circ$, Mutant bacteria (*kdsB*) with temperature-sensitive CMP-KDO synthetase at the non-permissive temperature (42 °C); $\square$, wild-type pathogen with 50 μ g ml⁻¹ compound IV; Δ , wild-type pathogen with no drug. *S. typhimurium* LT2, strain RG111 (a wild-type pathogen) was grown in MOPS defined medium¹³ containing 0.2% glucose (v/v) and 0.5 mM *N*-acetyl-D-glucosamine. At an A₄₂₀ value of 0.3, 50 μ g ml⁻¹ of IV was added simultaneously with 4 μ Ci ml⁻¹ ³H-*N*-acetyl-D-glucosamine. When growth stasis occurred (2–3 h) cells were collected and washed twice in 0.01 M HEPES-NaOH buffer, pH 7.4. The cell pellets were suspended in 1.25 ml of methanol containing 0.1 N HCl, and 0.5 ml of H₂O and 0.625 ml of chloroform were added. After vigorous mixing, samples remained at room temperature for 20 min. Water (0.625 ml) and chloroform (0.625 ml) were added, samples mixed, and then centrifuged at 5,000 r.p.m. (Sorval SS-34 rotor) for 10 min at 4 °C. The lower chloroform phase was removed, and 1.25 ml of chloroform were added to the upper methanol-H₂O phase, to repeat partitioning of precursor into chloroform. The combined chloroform phases were dried under N₂, resuspended in chloroform, and applied to Silica Gel H. Thin-layer plates were developed for 2 h in chloroform/pyridine/88% formic acid/water (40:60:16:5, v/v). Lanes were scraped into 0.5-cm fractions which were counted for radioactivity. The same method was used for analysis of the *kdsB* mutant, except for use of a temperature shift (30 to 42 °C), rather than drug, to inhibit the KDO pathway. Precursor peak 4 was identified as species IV_A (for notation see refs 12 and 13) by comigration. The other three more polar (slower migrating) precursor components represent derivatives of IV_A which contain either phosphoethanolamine, or an aminodeoxypentose, or both^{12,13}.

The three peptide permease systems genetically defined in *S. typhimurium* are the oligopeptide (*opp*), tripeptide (*tpp*) and dipeptide (*dpp*) permeases¹⁴. Antibacterial activity and associated physiological and biochemical effects required an intact oligopeptide permease system, and little if any transport occurred by the tripeptide or dipeptide permease systems (Table 1). Antibacterial activity of two peptide prodrugs, IV and V, also required either aminopeptidase A (*pepA*) or N (*pepN*) activity, whereas a third, VI, was still partially active in the absence of these two peptidases (Table 1). We conclude that compound IV enters bacteria by the *opp* system and is cleaved by specific aminopeptidases to effect release of the inhibitor (compound III); this is supported by the fact that inhibitory activity against purified CMP-KDO synthetase is abolished by attachment of a single amino acid, or dipeptide to the N-terminus of compound III.

The metabolic fate of IV in cell-free extracts was monitored by HPLC (C18 column) analysis of metabolites derivatized with phenylisothiocyanate, in order to elucidate the pathway and rate-limiting step in release of inhibitor from the peptide prodrug. The N-terminal alanine of compound IV is released rapidly by one of several aminopeptidases, including A and N. In contrast, the alanine residue linked directly to compound III is released more slowly and is dependent on peptidase A or N activity. These data, implicating aminopeptidase cleavage of the amino acid-inhibitor bond as the rate limiting step in inhibitor release, are supported by the following observations. First, rates of inhibitor release from compounds IV and VII (the latter contains only a single alanine linked to III) were identical (33

Table 1 Antibacterial activity of peptide prodrugs of III

Bacterial strain	Relevant genotype*	Zone of inhibition (mm) caused by 1 μ mol of compound			
		III	IV	V	VI
RG 111	Wild-type pathogen	0	29(33)†	34(150)†	24(17)†
RG 143	<i>oppB255::Tn10</i>	0	0	0	0
RG 166	<i>dpp101::Tn5</i>	0	29	35	24
RG 144	<i>tppB16::Tn10</i>	0	29	34	25
RG 165	<i>tppB16::Tn10</i>	0	29	34	25
	<i>dpp101::Tn5</i>				
RG 160	<i>pepA201::Tn10</i>	0	25	27	20
RG 171	<i>pepN88::Tn10</i>	0	22	21	32
RG 177	<i>pepA201::Tn10</i>	0	0	0	10
	<i>pepN88::Tn10</i>				

Bacteria were plated in soft agar on MOPS glucose minimal medium¹⁶ and a 6-mm paper disk containing compound was applied to the agar surface. Zones of growth inhibition (diameter) were recorded after 24 h at 37 °C.

* Peptide transport and peptidase alleles were from C. Higgins¹⁴ and C. Miller¹⁷ respectively. Mutant alleles were moved into strain RG 111 by phage P22 transduction, yielding strains 143, 144, 160, 165, 166, 171 and 177 which are thus isogenic. A tetracycline-sensitive variant of strain 160 was selected, allowing introduction of the *pepN88::Tn10* lesion to give the double mutant 177.

† The value in parentheses is the rate of release of inhibitor III from the peptide prodrugs. These data were determined in toluenized cells as described¹⁸. The rates 17, 33 and 150 pmol per min per mg protein for VI, IV and V, respectively, were correlated with their antibacterial potencies (zone of growth inhibition).

Table 2 Inhibition of cell growth by compound IV renders bacteria susceptible to serum killing

Pretreatment	% Survival following treatment with 50% human serum for 40 min at 37 °C
None	113 ± 16.2
Chloramphenicol (50 µg ml ⁻¹)	98 ± 2.9
Erythromycin (750 µg ml ⁻¹)	86 ± 2.3
Tetracycline (5 µg ml ⁻¹)	94 ± 9.8
Compound IV (100 µg ml ⁻¹)	1.3 ± 0.2*

Cells were grown in MOPS medium containing 0.2% glucose (w/v) and pretreated as indicated. Drug concentrations in the pretreatment were chosen so that growth ceased in 2–3 h. An equal volume of phosphate-buffered saline (PBS) or normal human serum was added, and samples incubated at 37 °C for 40 min. No loss of viability occurred in PBS. Survival values in serum are given with the standard deviation as determined by viable count in triplicate.

* Most (>95%) of the bacteria were killed in 20 min and addition of fresh serum at 40 min resulted in continued killing, that is, serum complement is exhausted when large numbers of bacteria are used.

pmol per min per mg protein at 100 µM substrate). Second, release of inhibitor from IV, V, VI and VII was inhibited by the aminopeptidase inhibitor bestatin; and third, there was a correlation between inhibitor release in cell extracts and antibacterial potency (Table 1).

Inhibition of LPS synthesis, accumulation of lipid A precursor, and bacteriostasis, however, are not the final consequences of treatment with compound IV. Translocation of drug-induced lipid A precursor to the outer membrane (S. Kadam *et al.*, in preparation) causes development of sensitivity to host defences and increased sensitivity to antibiotics known to have difficulty crossing the outer membrane permeability barrier. When compared to other bacteriostatic agents, induction by compound IV of susceptibility to complement killing is striking (Table 2). No killing is observed following bacteriostasis induced by tetracycline, chloramphenicol, or erythromycin; however, extensive killing results from pretreatment with IV. In addition, cells become 10 times more sensitive to erythromycin following pretreatment with IV, such that 50% inhibition of protein synthesis rate is brought about by 10 µg ml⁻¹ rather than 100 µg ml⁻¹ erythromycin.

In conclusion, peptide prodrugs of compound III represent antibacterial agents which specifically inhibit LPS synthesis at the site of KDO metabolism. Previously, diazaborine was considered to act in this manner¹⁵, but further studies revealed that this was not the case and suggested that its antibacterial action was due to inhibition of fatty acid biosynthesis (K. Fuchs and G. Hogenauer, personal communication). We have confirmed these results, finding that synthesis of all classes of fatty acid are inhibited by diazaborine, probably by inhibition of acetyl CoA carboxylase (unpublished data).

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A liver-stage-specific antigen of *Plasmodium falciparum* characterized by gene cloning

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The liver phase of development of malaria parasites has been studied only recently and remains poorly understood compared to the other stages such as sporozoites, merozoites and gametes^{1,2}. Access to liver forms of *Plasmodium falciparum* has been improved by the development of *in vivo*³ and *in vitro*⁴ propagation methods, but the yield of mature schizonts remains limited and does not allow a detailed antigenic analysis. To date, only immunofluorescence assays (IFA) have permitted a description of a species and liver-stage-specific antigen(s) (LSA; ref. 3). Monospecific antibodies to these antigens have not been obtained due either to difficulty in immunizing mice (against LSA), or to poor stability of human monoclonal antibodies. Therefore, as a means of characterizing the LSA, we used an alternative immunological approach to identify clones of the corresponding LSA genes. We describe here the isolation of a DNA sequence coding for a *P. falciparum* liver-stage-specific antigen composed of repeats of 17 amino-acids, which is immunogenic in man.

We looked for human sera with restricted specificity to the pre-erythrocytic stages of development of *P. falciparum* by screening individuals living in a malaria endemic area and undergoing continuous drug prophylaxis. One such serum taken from a subject ingesting 100 mg of chloroquine daily, whilst being under continuous exposure to malaria for 26 years, had high antibody titres to sporozoites (1/3,200 by IFA) and liver stages (1/6,400) yet was essentially negative with blood-stage parasites (<1/100). The serum was used to screen a genomic expression library of *P. falciparum* Tak9.96 DNA⁵ cloned in λgt11 (ref. 6). The recombinant phages were first screened with a pool of hyper-immune sera and 2,000 antigen-producing clones were detected. Of these only 15% were positive with the sporozoite and liver-stage-restricted serum. This indicates that the antibodies to sporozoite- and liver-stage antigens are a restricted subset of the total antibody responses to malaria parasites. Of these clones the 22 most positive were selected for further study. Human antibodies from the original serum were affinity-purified on β-galactosidase fusion proteins from these clones⁷. The stage specificity was assayed by IFA using *P. falciparum* sporozoites, liver- and blood-stage parasites. Antibodies selected on three clones (DG145, DG199 and DG307) reacted specifically with liver schizonts: location of the fluorescence was very similar to that considered characteristic of LSA (ref. 3; Fig. 1). The reaction was *P. falciparum*-specific as the affinity-purified antibodies were negative with *P. vivax* liver schizonts prepared in *Saimiri* monkeys and with *P. yoelii* liver stages grown in BALB/c mice. Moreover, the three recombinant antigens were negative with sera to *P. vivax*, *P. ovale*, *P. cynomolgi* and *P. yoelii* (data not shown).

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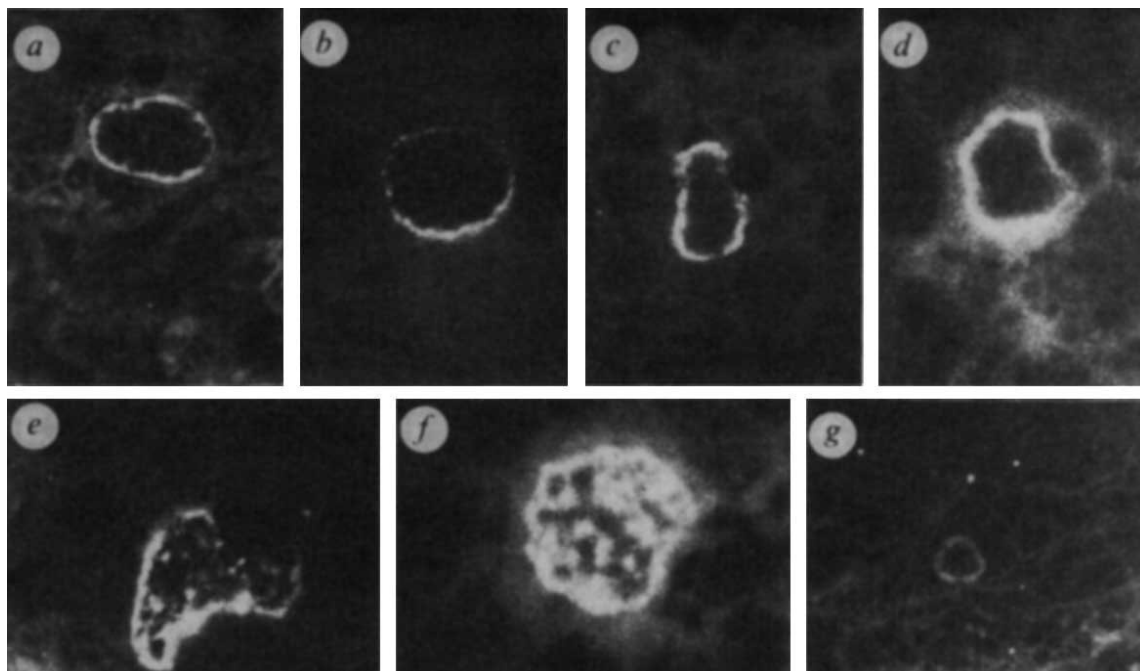


Fig. 1 Reaction and localization of antibodies with liver-stage schizonts. *a*, Typical immunofluorescence using adult African serum diluted 1/2,000 and reacted with 5- μ m sections of Carnoy fixed liver fragments taken from *Cebus apella* monkeys infected with *P. falciparum* (ref. 3). Same antigen reacted with antibody eluted from protein expressed by: *b*, clone DG307; *c*, clone DG145; *d*, clone DG199; *e, f*, the same as *a* and *b*, using more mature schizonts to show the internal distribution of the antigen. *g*, Liver schizonts reacted with 1/250 dilution of a rabbit serum raised to clone DG307 fusion protein (one i.m. injection with FCA of the recombinant fusion protein isolated by preparative gel electrophoresis followed by four additional i.v. injections at 15-day intervals).

The recombinant fusion proteins of DG145, DG199 and DG307 were positive with the original human serum (Fig. 2*a*) and with ten other African sera, but negative with sera from transfusion malaria patients and anti-sporozoite sera (data not shown). The three fusion proteins were found to be heat-stable as the LSA epitopes remained antigenic after boiling at 100 °C for 15 min. This is shown for DG307 in Fig. 2*a* track 2. Full immunological cross-reactivity between the three recombinant antigens was demonstrated by immunoblot analysis of the fusion proteins. Antibodies affinity-purified on the DG307 protein reacted to the same extent with the recombinant antigens of clones DG307, DG145 and DG199, but they did not react with the recombinant antigens of unrelated clones. The same cross-reactivity was also observed using affinity-purified antibodies corresponding to clones DG145 and DG199 (data not shown).

The liver-stage-specificity of LSA was further established by raising a polyclonal monospecific antiserum to the DG307 fusion protein. The anti-DG307 rabbit serum gave the characteristic LSA immunofluorescence image (Fig. 1*g*). Like the affinity-purified human antibodies selected using the three clones, this serum was negative by IFA and immunoblot with sporozoites and blood-stage antigens (Fig. 2*b*).

To analyse the relationship between clones and to characterize the LSA gene(s), DNA from the three LSA phages DG145, DG199 and DG307 was prepared and the individual *P. falciparum* DNA inserts recloned into the plasmid pUC9 and bacteriophage M13 (ref. 8). DG145 contained two *P. falciparum* DNA fragments of 400 and 700 base pairs (bp) (Fig. 3*a*). The 700-bp fragment cross-hybridized with the 196-bp insert in phage DG307, but did not cross-hybridize with the 600-bp fragment of DG199. Genomic DNA was cut with four different restriction enzymes (both single and double digests) and was used in a series of Southern transfer analyses (one example is shown in Fig. 3*b*). The analysis indicated that the DG307, DG199 and DG145 700-bp fragments were derived from the same region of the genome as they all hybridized to a *Rsa*I fragment of 2.2 kilobase (kb), a *Dra*I fragment of 4.5 kb and an *Alu*I fragment

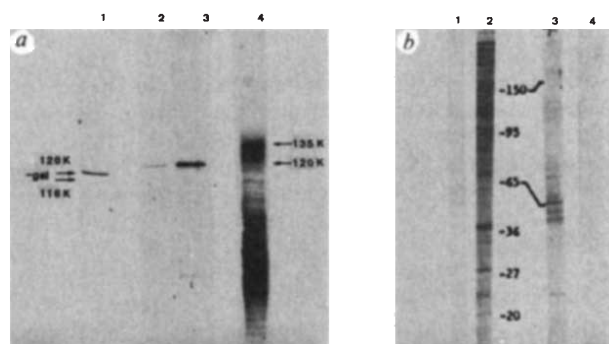


Fig. 2 *a*, Identification of the fusion proteins of the three clones expressing LSA antigen by human antibodies. SDS-PAGE electrophoresis of proteins from cultures of DG199 (track 1), DG307 (tracks 2 and 3) and DG145 (track 4). *b*, Stage specificity of DG307. Rabbit antibodies to DG307 (1/20 dilution) did not react on immunoblots with antigens extracted from blood stages (track 1) and sporozoites (track 4). Shown as a control is an immune African serum (1/100 dilution) which is positive with both erythrocyte (track 2) and sporozoite (track 3) stage antigens. Note that the recombinant protein of DG307 remains antigenically reactive after boiling and also that the fusion protein of DG145 appears somewhat degraded.

Methods. The three LSA clones were isolated from a genomic expression library constructed in the following way. Random fragments of T9.96 DNA were generated by the action of DNase1, methylated to protect the endogenous *Eco*RI sites and cloned by the addition of linkers into the *Eco*RI site of λ gt11. The fusion protein of DG307 was boiled in PBS at 100 °C for 15 minutes before gel electrophoresis (track 2). Proteins were transferred to nitrocellulose filters according to Biorad recommendations and antigenically reactive proteins detected by incubation with 1/100 dilution of the selective immune human serum, or 1/20 dilution of rabbit serum depleted of anti β -galactosidase and anti *Escherichia coli* antibodies. Immune complexes were revealed using anti-human and rabbit IgG, A, M peroxidase labelled antibody (Biosys, diluted 1/500) and diaminobenzidine substrate.

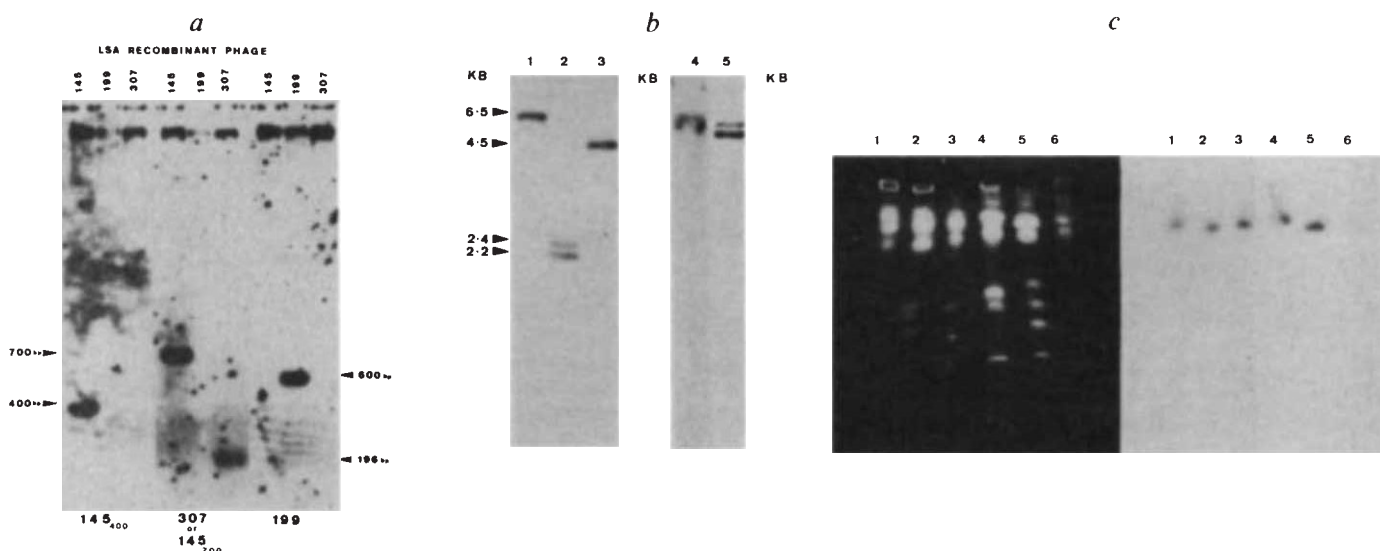


Fig. 3 Analysis of the LSA gene. *a*, Analysis of *P. falciparum* DNA inserts in the recombinant LSA phages. DNA was prepared from DG145, DG199 and DG307, restricted with *EcoRI* and size fractionated on a 1% agarose gel. The DNA was transferred⁹ to Hybond-N according to Amersham protocol and hybridized at very low stringency ($2\times$ SSC, 37°C) with the radiolabelled pUC9 plasmid recombinants containing separately each of the *EcoRI* fragments derived from the LSA recombinant phages. The 600-bp sequence of DG199, like the 400-bp insert of DG145, hybridized only to itself whereas the 700-bp DG145 insert cross-hybridized to the 196-bp fragment contained in DG307 (and vice versa), indicating that they contain homologous sequences. *b*, Parasite DNA restriction fragments hybridized with DG307. Track 1, *EcoRI*; 2, *RsaI*; 3, *DraI* acting on Tak9.96 DNA. Tracks 4, *EcoRI*; 5, *RsaI* acting on Palo Alto DNA (FUP Uganda). The restricted DNA was transferred⁹ to Hybond-N (Amersham) and hybridized under stringent conditions ($0.5\times$ SSC, 65°C). Note that two *RsaI* fragments of different size are identified in Palo Alto DNA. *c*, Chromosomes separated by pulse field gradient electrophoresis^{10,11}. The karyotypes presented are for strains (1) Palo Alto (2) D3, (3) 7G8, (4) B9, (5) C11 (6) Tak9.96. B9 and C11 are clonal derivatives of a single Thai isolate (P.D., unpublished) and D3 is a clonal derivative of FCR3 (a kind of gift of W. Trager). The gel (1% agarose) was run at 250 V using a 75-s pulse for 20 h. The variation in size is due to chromosome polymorphism^{12,13}. The chromosomal gel was transferred⁹ to Hybond-N and probed with the inserts of DG145, DG199 and DG307 cloned in pUC9 and radiolabelled. The same large, poorly separated chromosome (number 6 counting from bottom) was identified in all strains by clones DG307, DG145 and DG199; the result obtained with plasmid recombinant DG307 is shown.

of 1.3 kb. The observation that antibodies to the DG199 fusion protein cross-reacted with the fusion proteins of DG307 and DG145, whereas its nucleotide sequence differs from the 51-bp repeat, suggests that DG199 encodes a different type of repetitive cross-reacting peptide. This situation has already been observed for several other *P. falciparum* blood-stage antigens such as FIRA and RESA^{15,16}.

The LSA locus shows restriction polymorphism for the enzymes *RsaI* and *AluI* as DNA fragments of different size were found when other strains were examined. Figure 3b shows the result obtained with *RsaI* and the *P. falciparum* Ugandan isolate Palo Alto (compare tracks 2 and 5). Karyotype analysis demonstrated that the three LSA clones are located on one of the large chromosomes and were found to be conserved in all the *P. falciparum* strains examined (Fig. 3c). Consistent with this observation is that the clones were derived from a Thai parasite and were selected by human sera from Africa. Finally, no sequences homologous to the *P. falciparum* LSA clones were found in heterologous species by probing the genomes of *P. vivax* and *P. chabaudi* (data not shown).

In order to allow further immunological studies and to complement the rabbit LSA-specific serum raised to the DG307 β -galactosidase fusion protein, we generated the corresponding synthetic peptide. The DNA sequence of DG307 was determined and a synthetic peptide prepared. Figure 4 shows that clone DG307 contains a DNA fragment of 196 bp composed entirely of a 51-bp repeat. Only one reading frame is in frame with the β -galactosidase *lacZ* gene and the clone produces a fusion protein that carries the epitopes recognized by the human sera (Fig. 2). The inferred amino-acid sequence corresponding to this frame is also presented. It is composed of a 17-residue repeat rich in glutamine, glutamic acid and leucine. Unlike the DNA sequence, the amino-acid sequence of the repeats is highly conserved. The one change in the third repeat results from an A to G substitution at the second position in the eighth codon.

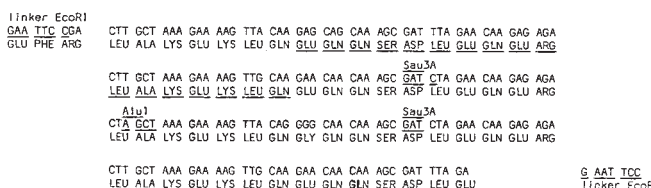


Fig. 4 The DNA and amino-acid sequence of DG307. DNA sequence of the 196-bp *P. falciparum* genomic DNA fragment expressed in clone DG307. The *EcoRI* linker is underlined, as are some restriction sites. Note that the repeats are not perfectly conserved. Substitution of an A for a T creates an *AluI* site in the third repeat. A change from a T to a C gives *Sau3A* sites in the second and third repeats. None of these changes result in an amino-acid substitution. The sequence overall is 61% A + T. Shown below the DNA sequence is the amino-acid sequence expressed by clone DG307. The amino acids corresponding to the *EcoRI* linker are also given as they denote the reading frame. The first arginine and last glycine are therefore artificial as they are encoded in part by the linker and in part by *P. falciparum* DNA. Shown underlined are the amino acids of the synthetic peptide used to confirm the sequence. The 196 bp of *P. falciparum* DNA of DG307 were excised by digestion with *EcoRI*, cloned in both orientations into the *EcoRI* site of the single-stranded phage m13mp8 and sequenced by the chain-termination method¹⁴.

Otherwise all other substitutions are silent. No homology with known DNA and protein sequences was detected (at the 60% level) when the Los Alamos and NBRF data banks were screened. The peptide (EQQSDLEQERLAKEKLG) corresponding to the amino-acids underlined (see Fig. 4) was synthesized and used in ELISA assays. Its reactivity with the rabbit anti-DG307 serum confirmed the deduced DNA sequence. The synthetic peptide reacted equally with the human antibodies affinity-purified on the fusion proteins of clones DG307, 145 and 199. This emphasizes the antigenic similarity of the three

recombinant antigens. Moreover, the reaction of the synthetic peptide with the serum used in the initial screening, together with its reaction with ten other African sera indicate that a single 17 amino-acid repeat carries at least one epitope corresponding to an antibody specificity in human sera.

The 17-amino-acid repeat described here is the first such reported for a malaria antigen. As repeated epitopes have been described for several *P. falciparum* antigens¹⁷ it is not surprising that LSA, which is immunogenic in humans, also possesses repeated structures. Computer analysis predicts that, in contrast to the CS protein, the LSA repeat with seven or eight charged amino acids may assume a helical structure. The observation that the amino-acid sequence is more highly conserved than the DNA sequence argues for a functional or structural role for these repeats. Finally the above results, obtained using a novel approach to a poorly accessible stage, provide the first data on the structure of a protein specific for the liver stage of development of *P. falciparum*. Availability of recombinant antigens, synthetic peptides and the corresponding antibodies now provide a way to evaluate the role and biological function of this stage-specific protein.

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Involvement of the *pumilio* gene in the transport of an abdominal signal in the *Drosophila* embryo

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A small set of maternal genes controls the basic longitudinal pattern of the *Drosophila* embryo. For the posterior pattern, five genes are necessary both for abdominal segmentation and for pole cell formation^{1–3}. Cytoplasmic transplantations involving *oskar* (*osk*) mutant embryos have suggested that the pole plasm serves as a source of a signal required in the more anterior abdominal region³. Here we present evidence that the maternal gene *pumilio* (*pum*) is involved in the transport of this signal to the abdominal region. In *pum* embryos (that is, embryos from females of genotype *pum/pum*) only the abdomen is affected, whereas the pole plasm seems normal. Transplantation experiments reveal that *pum* pole plasm contains the abdominal signal but that it cannot reach the target site, the abdomen. Abdominal segmentation is restored when the physical separation between pole plasm and abdominal region is overcome either by transplantation of *pum* pole plasm into the abdominal region of *pum* embryos or by genetic means in double mutants with *torsolike* (in mutant *torsolike* embryos the abdominal region is juxtaposed to the pole plasm).

Embryos derived from females homozygous for a strong *pum* allele form no more than two of the normal eight abdominal segments whereas head, thorax and the posterior end (the telson) are normal (Fig. 1b). A similar phenotype has been described for the zygotic mutant *knirps* (ref. 4) and for five maternal effect loci: *tudor*, *vasa*, *staufen*, *valois* and *oskar* (refs 1–3). In contrast to these maternal mutants, *pum* embryos do form pole cells, the germ-line precursors. These pole cells are functional when transplanted into otherwise sterile host embryos, such as the progeny of *osk*³⁰¹/*osk*³⁰¹ females⁵. Morphologically, we cannot detect a difference between *pum* and wild-type pole plasm⁵. Polar granules⁶ are present. Thus, the embryonic function of the *pum* gene product is restricted to the development of the abdomen.

We tested whether the abdominal phenotype of *pum* embryos could be rescued by transplantation of wild-type cytoplasm.

Using our standard injection procedure³ the abdominal phenotype of *pum* embryos can be partially rescued by transplantation of cytoplasm from wild-type donors into the abdominal region of *pum* recipients (Fig. 2a). However, unlike in mutant *osk* embryos, completely normal abdominal segmentation is not restored. In mutant *pum* embryos the rescuing activity does not seem to spread but acts directly at the site of injection. For example, injections into the dorsal side of the prospective abdomen lead to the development of dorsal structures and leave the ventral side strongly mutant (Fig. 1c). Injections anterior and posterior to the abdominal region (20–50% egg length, where 0% is the posterior pole) rarely lead to a rescue (Fig. 2a). Despite this specificity of *pum* for abdominal development, rescuing activity is recovered only from the posterior pole of donor embryos: homotopic transplantation of abdominal cytoplasm into the abdomen of *pum* embryos does not lead to any change in phenotype (Table 1). Thus, although the mutant phenotype of *pum* suggests independence of abdominal development and pole cell formation, the transplantation experiments show identical localization of rescuing activity for *pum* and *osk* to the posterior pole. This may indicate that the abdominal defects in *pum* and *osk* embryos are caused by the lack of the same signal.

To test whether the activity that rescues *pum* embryos is present in *osk* embryos, we transplanted cytoplasm from *osk* embryos into *pum* embryos. No rescue occurred (Table 1), indicating that the repair of the *pum* mutant phenotype is dependent upon *osk*⁺ activity. In the reciprocal experiment (transplantation of *pum* pole plasm into the abdominal region of *osk* embryos) the *osk* phenotype is rescued to the same extent as after transplantation of wild-type cytoplasm (Fig. 2b). One explanation for this finding is that *pum* embryos possess a functional signal, but that the signal is restricted to the pole plasm region and is therefore unable to reach the abdominal region. In which case *pum* pole plasm should rescue the *pum* phenotype.

Injection of posterior pole plasm from *pum* embryos into the abdominal region of *pum* embryos does indeed restore abdominal segmentation (Table 1). As in the wild type, rescuing activity is recovered only from the posterior pole region (0–20% egg length). Thus, *pum* and *osk* may lack the same signal in the abdominal region: the signal cannot be detected in *osk* embryos; it is present in *pum* embryos, but is trapped at the posterior pole. If this interpretation is correct, the distribution of the signal might be altered in *pum* embryos. We compared the spatial distribution of rescuing activity in wild-type and *pum* donors. In earlier experiments, when about 5% egg volume was

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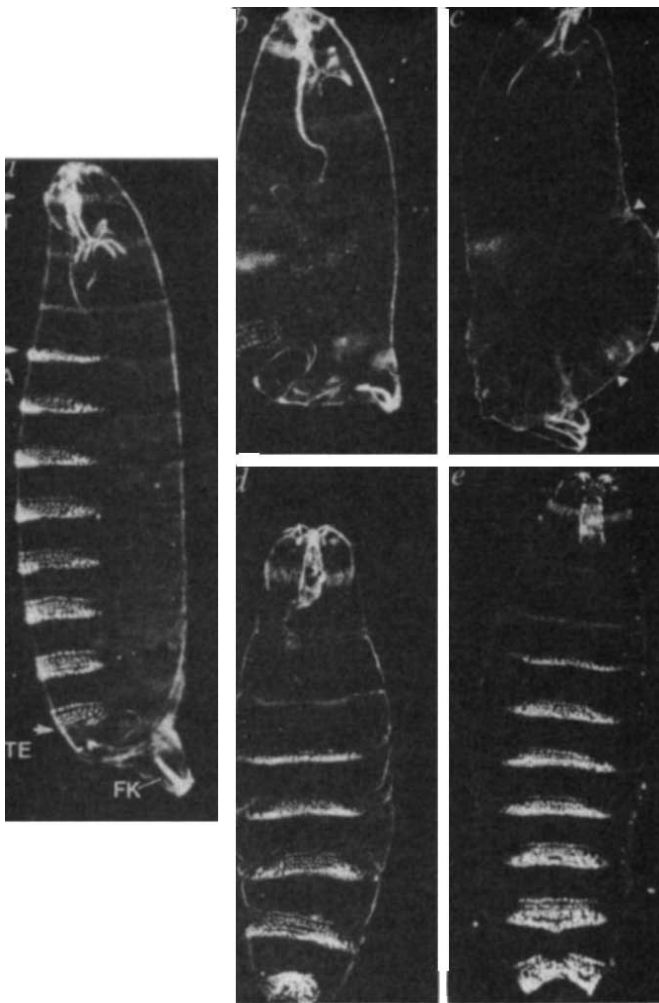


Fig. 1 Dark field photographs of cuticular preparations of *pumilio* mutant embryos. *a*, Wild type. For detailed description of the wild-type pattern see refs 14–16. T, thoracic region; A, abdominal region; TE, telson; FK, Filzkörper. *b*, Strong *pum* phenotype (maternal genotype: *pum*⁶⁸⁰/*pum*⁶⁸⁰; 18 °C). Head and thorax are normal, in this embryo two abdominal segments are formed. *c*, Locally restricted response of *pum* embryo upon injection. Posterior pole plasm of embryos derived from homozygous *pum*⁶⁸⁰ females was injected into the dorsal side of a *pum*⁶⁸⁰ embryo at ~40% egg length (0% represents the posterior pole). Dorsally five abdominal segments are formed (◄) whereas the ventral side continues to show the mutant phenotype. *d*, The *pum* *tsl* double-mutant embryo. The effect of *pum* on abdominal development is partially suppressed and five abdominal segments are formed at the restrictive temperature of 18 °C. The same phenotype is observed for *tor*; *pum* and *pum* *tsl* double mutant embryos. This phenotype is in contrast to double mutants between *fs(1)Nasrat*, *tor* or *tsl* and other members of the posterior-group genes, such as *vasa*, *tudor*, *oskar*. In such embryos simple addition of phenotypes is observed^{2,5,7}. *e*, The *tsl* phenotype. Embryos derived from homozygous mutant *tsl* females lack the most anterior and posterior structures. Anteriorly, the labrum is missing and the cephalo-pharyngeal skeleton is reduced and posteriorly the eighth abdominal segment and the telson are deleted (H. G. Frohnhöfer, unpublished data). Note that in *tsl* embryos seven abdominal segments are formed whereas *pum* *tsl* double-mutant embryos develop on average no more than five abdominal segments. *a–c*, Side view; ventral left, anterior up. *d*, *e*, Frontal view on ventral side, anterior up.

injected per *osk* recipient, we were unable to detect any activity in wild-type donors anterior to 10% egg length³. However, when each recipient is injected with a cytoplasmic fraction pooled from 2–3 donors (about 15% egg volume), some activity can be recovered anterior to the pole region of wild-type embryos up to 30% egg length. There is some indication that the rescuing activity in *pum* embryos is more closely restricted to the pole than in wild type (Fig. 2*b*). Such a difference in distribution is expected if mutations in *pum* affect a general transport mechanism. The same mechanism could also be responsible for the

distribution of activity at the target site, as suggested by the local response of the recipient *pum* embryos upon transplantation, but other explanations are equally possible.

The defect in signal transport by mutations in the *pum* locus can be partially overcome by genetic means. In embryos derived from females mutant for one of the genes of the 'torso group': *torso* (*tor*, ref. 2), *trunk* (ref. 2), *fs(1)Nasrat* (ref. 7) and *torsolike* (*tsl*, H. G. Frohnhöfer, personal communication), the fate map at the posterior is altered such that the anlagen of the abdomen and the pole plasm are no longer separated by the telson but

Table 1 *pum* rescuing activity in wild-type, *osk*, and *pum* donor embryos

Maternal genotype of donor	Position in donor (% egg length)	Developed (N)	Recipient <i>pum</i> embryos Abdominal segments formed (%)		
			none	1 or 2	3 and more
Wild-type	0–10	77	16	43	41
Wild-type	10–20	52	47	47	6
Wild-type	30–40	26	39	61	0
<i>pum</i>	0–10	79	20	47	33
<i>pum</i>	10–20	28	21	76	3
<i>pum</i>	30–40	31	67	23	0
<i>osk</i>	0–10	41	53	47	0
Controls					
<i>pum</i> uninjected		120	73	27	0
<i>pum</i> pricked		22	69	31	0

Cytoplasm of donor embryos taken from the position indicated was injected into the abdominal region (20–50% egg length) of *pum* embryos. For controls we used either uninjected embryos or embryos which were dried in parallel to the experimental animals and then pricked at 30% egg length during early cleavage. We considered as rescued only those embryos which developed more than two abdominal segments, because some of the control embryos developed one or two abdominal segments. This may lead to an underestimate of the overall frequency of rescue.

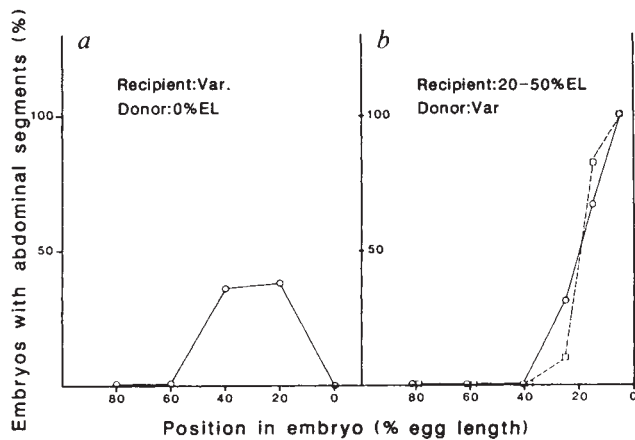


Fig. 2 Spatial distribution of rescuing activity in wild-type and *pum* embryos and its requirement for abdominal segmentation in *pum* and *osk* embryos. The restoration of abdominal segmentation after injection was measured by the percentage of embryos which developed more than two (injections into *pum* embryos) or between one and eight abdominal segments (injections into *osk* embryos). *a*, Wild-type posterior pole plasma was injected into different positions in recipient *pum* embryos. *b*, Results of using *osk* embryos as recipients for *pum* and wild-type cytoplasm. Cytoplasm from different positions in wild-type (○) and *pum* (□) embryos was injected into the abdominal region of *osk* embryos. In these experiments each recipient was injected with cytoplasmic fractions collected from 2-3 donors, which corresponds to an injection of ~15-20% egg volume. Similar results have been obtained with mutant *nanos* (*nos*) embryos as recipients for *pum* and wild-type cytoplasm (data not shown). Like *osk* and *pum*, *nos* is a posterior-group gene (R.L. and C.N.V., manuscript in preparation).

Methods. Injections were carried out as described³ except that on average more cytoplasm per embryo was transplanted. Each data point represents on average 25 differentiated embryos in *a*, and on average 100 embryos in *b*, which were collected in several independent experiments. The *pum* embryos were dried on silica gel and kept at 18 °C throughout development to avoid phenotypic variations due to the temperature-sensitivity of *pum*. Oregon R was the wild-type stock. For injections, embryos from females homozygous for the allele *osk*¹⁶⁶ or the allele *pum*⁶⁸⁰ were used. VAR, variable position; EL, egg length.

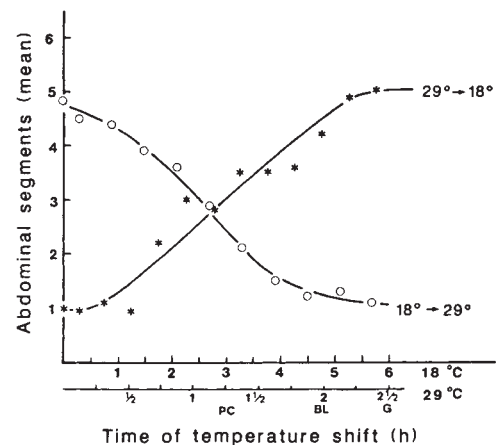


Fig. 3 Temperature-sensitive period of *pum*. Each measurement indicates the number of abdominal segments formed by embryos which at the time of the shift had reached the age indicated (PC, pole cell formation, stage 3; BL, cellular blastoderm, stage 5; G, gastrulation, stage 6, ref. 17). The temperature-sensitive period of *pum* is after egg deposition. Before egg deposition we cannot detect any alteration in phenotype at the restrictive temperature. However, we observe a slight improvement of the embryonic phenotype when females are shifted to 29 °C. After several days the progeny of such females will on average form more abdominal segments than embryos which were shifted from the restrictive to the permissive temperature immediately after egg deposition.

Methods. Eggs were collected on yeast agar/apple juice plates for 6 h at 18 °C and 3 h at 29 °C. Embryos were shifted to 29 °C (shift up, ○) and 18 °C (shift down, *) respectively and covered with Voltalet 3S oil at the appropriate temperature. Embryos which had just started to gastrulate were collected every 15 min for the shift up and every 30 min for the shift down experiment. Such embryos can be recognized by the appearance of the ventral furrow and the headfold. When embryonic development was completed and the embryos had formed a well differentiated cuticle (48 h at 18 °C and 16 h at 29 °C), cuticular preparations were made and the number of abdominal segments was determined. Each data point represents the average number of segments formed ($N = 100$ embryos scored per point in three independent experiments each).

become juxtaposed. Thus the cells which would normally give rise to the posterior end of the larva (between 5-15% egg length) now develop abdominal structures (Fig. 1e). In the progeny of females mutant for both *tsl* and *pum*, the effect of *pum* on development is partially suppressed and several abdominal segments develop (Fig. 1d). A simple explanation for this result is that the telson provides a discrete barrier and its removal in *tsl* mutant embryos allows for signal transport in the absence of *pum* function. Alternatively, as discussed above, *pum* may fulfil a more general function not restricted to the telson and signal transport is no longer essential for abdominal development simply because source and target are now partially overlapping.

Mutant *tailless* (*tll*) embryos have a phenotype similar to *tsl*⁸ and segmentation in *tll pum* double-mutants is restored in a similar way as in double mutants between *pum* and *tsl*, but *tll* is a zygotic gene. A shift in fate map caused by a zygotic mutant can thus partially overcome the *pum* phenotype. This suggests that the signal transmitted by *pum* is interpreted at the time when the embryonic genome is active. These findings correlate quite well with the late phenocritical period (Fig. 3) of *pum* function during early embryogenesis (0-4 h after egg deposition). The phenotypes of all *pum* alleles and all alleles in *trans* to a deficiency of the locus are cold-sensitive. Embryos kept at 18 °C show a strong mutant phenotype, whereas those at 29 °C can form up to the normal number of abdominal segments. We do not know whether the cold-sensitivity is due to reduction of gene function or whether it represents the lack-of-function

phenotype⁵. It is possible that during early embryogenesis *pum*⁺ activity is required to stabilize an otherwise cold-sensitive process which promotes the transport of the abdominal signal from its source at the posterior pole to its target site in the prospective abdominal region. Microtubules are known to be major components needed for intracellular transport⁹ and are unstable at low temperatures *in vitro*¹⁰. While *pum* is not one of the identified *Drosophila* tubulin genes¹¹ its product may be associated with the tubulin network.

The function of the *pum* gene product is not restricted to early embryogenesis. In our initial screen for maternal effect mutations one *pum* allele was recovered (C.N.V., G. Jürgens, K. V. Anderson and R.L., unpublished data). We isolated a further twelve alleles on the basis of non-complementation for the maternal phenotype (R.L., H. G. Frohnhofer, K. V. Anderson, U. Mayer and C.N.V., unpublished data). Eleven of these alleles in *trans* to a deficiency for the region (*Df(3R)p-XT9*, ref. 12) and in heterozygous combinations show a recessive zygotic visible phenotype. In mutant adult flies additional macrochaetae (postalar, dorsocentral and scutellar bristles) form on the dorsal thorax and viability is reduced. This phenotype seems not to be temperature dependent. Transcription of the *pum* gene may thus occur at two separate times: maternally for the embryonic function and during larval or pupal development for imaginal disk development. The *pum* gene may not be transcribed at all during embryogenesis because the abdominal phenotype is independent of the embryonic genotype (no paternal rescue). We have

shown that the maternal *pum* product is not essential for the production of an abdomen specific signal but it is more likely to have a more general function in signal transport in the egg cell. The *pum* gene may be an example of a gene which is not exclusively required for a particular developmental process but is essential for its execution¹³.

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Variable stoichiometry of proton pumping by the mitochondrial respiratory chain

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The proton/oxygen stoichiometry and the mechanism of the proton pumping respiratory complexes in the mitochondrial respiratory chain have been central issues in bioenergetics for several years. Recently, a number of disagreements about stoichiometry have been resolved, and H^+/O ratios of 6 (refs 1-3) or perhaps 8 (refs 4 and 5) for succinate oxidation are now accepted. Suggestions that the stoichiometry in mitochondria is intrinsically variable ('slip' in the pumps) have been made⁶⁻⁹ but the evidence has been neither strong nor direct. We now show by direct measurement in steady-state conditions that the H^+/O ratio (measured as the charge/O ratio) for isolated mitochondria respiring on succinate varies from 6 at low membrane potential to 2.5-3 at membrane potentials of about 170 mV.

If the mitochondrial respiration rate is lowered by titration with a respiratory inhibitor the plot of protonmotive force (Δp) against respiration rate (J_o) is nonlinear^{6-8,10-12}. Two main explanations for this have been proposed: that the conductivity of the mitochondrial inner membrane to protons increases at high Δp (ref. 10), or that the stoichiometry of proton pumping by the respiratory chain decreases as Δp is raised⁶⁻⁸. It is possible to distinguish between these two explanations by measuring the stoichiometry of the mitochondrial respiratory chain at high values of Δp .

Most kinetic methods for assessing H^+/O ratios have been done at level flow, that is, conditions of vanishingly small Δp . One exception is the steady-state method of Al-Shawi and Brand¹³. Originally this was used to measure H^+/O ratios by measuring pH changes following perturbation of steady-state respiration. We have modified the technique to measure elec-

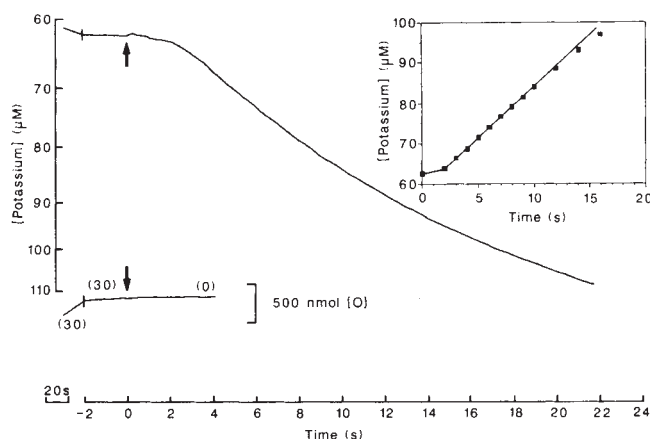


Fig. 1 Experimental determination of the q^+/O stoichiometry.

Experimental traces produced by the simultaneous measurement of oxygen and potassium concentrations with a potassium sensitive electrode (top trace) and an oxygen electrode (lower trace). J_o was measured from the slope of the oxygen trace immediately before the change in chart speed at time -2 s. J_{in} was determined from the rate of K^+ efflux following the addition of myxothiazol (arrows on traces). Because the potassium-sensitive electrode gives a logarithmic response to K^+ , the rate of efflux was determined from linearized replots as shown in the inset. Local slopes of the oxygen electrode trace are shown in parentheses. The scale bar for the oxygen electrode trace shows a concentration difference of 500 nmol. J_{in} was taken as the slope after ~ 2 s when the K^+ efflux had stabilized. There was no malonate present in this experiment. **Methods.** All experiments were carried out at 37 °C, using 2 mg of mitochondrial protein per ml in a well-stirred, closed, 10-ml system. Mitochondria were prepared from rat liver by standard methods¹⁷. The basic medium used contained 250 mM sucrose, 10 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonate (HEPES buffer), 5 mM phosphate, 1 mM ethyleneglycol bis (aminoethyl ether)-*N,N'* tetraacetate (EGTA), 100 μ M magnesium acetate, adjusted to pH 7.2 with tetramethylammonium (TMA) hydroxide. Further additions were 10 mM TMA succinate, 0.2 nmol valinomycin per mg mitochondrial protein, 5 μ M rotenone, 2.4 μ M carboxyatractylolide and 1.25 μ g ml⁻¹ oligomycin. The mitochondria were incubated in this medium for 5 min and then myxothiazol (2 μ M) was injected to stop respiration and start potassium efflux. The potassium-sensitive electrode was calibrated after the experiment by addition of known amounts of potassium chloride. The oxygen electrode was calibrated as described in ref. 18. The numbers on the oxygen trace are the rates of respiration in nmol oxygen per min per mg of mitochondrial protein.

trical charge/O (q^+/O) ratios by following K^+ movements. With electron transport from succinate to oxygen the q^+/O ratio will be equal to the H^+/O ratio.

Our method is based on the equality of the rate of charge efflux from mitochondria (J_{out}) to the rate of charge influx (J_{in}) in a steady state. The rate of charge efflux is related to the rate of respiration, J_o , by n , the value of the q^+/O stoichiometry for the substrate used, so that $J_{out} = nJ_o$. In a steady state $J_{out} = J_{in}$, and it follows that $J_{in} = nJ_o$. So, if we measure J_o and J_{in} for mitochondria respiring in a steady state we can determine n , the value of the q^+/O stoichiometry.

The experimental measurement of J_o and J_{in} is illustrated in Fig. 1. Mitochondria were incubated with succinate as substrate in a low-potassium medium containing valinomycin. In the steady state K^+ equilibrated with the mitochondrial membrane potential ($\Delta\psi$), and ΔpH was negligible. J_o was measured with a Clark electrode. The steady-state value of J_{in} was estimated using a potassium-sensitive electrode to measure the rate of K^+ efflux following inhibition of electron transport with myxothiazol. When myxothiazol was added respiration and J_{out} stopped, but it is assumed that J_{in} initially continued at its

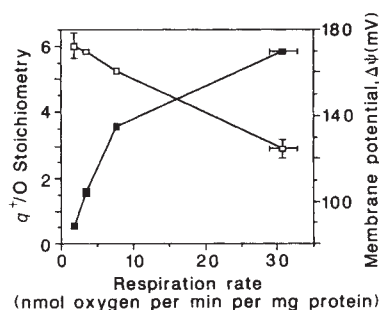


Fig. 2 Membrane potential (■) and q^+/O stoichiometry (□) plotted against respiration rate as it is lowered with the inhibitor malonate. These q^+/O stoichiometries were obtained by the method outlined in Fig. 1 and are plotted against J_o as it was lowered by different amounts of the inhibitor TMA malonate, from no malonate (highest value of J_o) to 12 mM malonate (lowest value of J_o). The membrane potential was determined in parallel from the distribution of $^{86}\text{Rb}^+$, which equilibrates with the potassium gradient. The membrane potential (in mV) is given by the Nernst equation; $\Delta\psi = 61.5 \log ([\text{Rb}]_{\text{in}}/[\text{Rb}]_{\text{out}})$.

Methods. Radioactive experiments were carried out in 1-ml plastic centrifuge tubes under the same conditions as for Fig. 1, but with the addition of $^{86}\text{RbCl}$ (20 nCi ml^{-1}) to measure $\Delta\psi$ and ^3H -sucrose (20 nCi ml^{-1}) as an indicator of the extra-mitochondrial pellet volume. The mitochondria were separated from the medium after a 5-min incubation by centrifugation in a bench-top centrifuge and the pellets and supernatants were separated and counted as described in ref. 19. The mitochondrial matrix volumes and ΔpH were determined in separate experiments as described in ref. 19. The volumes were about 0.6–0.8 μl per mg of mitochondrial protein. The pH gradients were in the range +10 mV to –10 mV and therefore ΔpH was considered to be small and constant and its contribution to Δp was ignored. The results for q^+/O and J_o are the means of two determinations and the results for $\Delta\psi$, ΔpH and mitochondrial volume are the means of six determinations. The error bars shown indicate the range of experimental results. Data from one typical day's experiment.

steady-state rate. The resultant tendency for $\Delta\psi$ to drop causes redistribution of K^+ across the membrane. Because of its large capacity, the K^+ gradient almost maintains $\Delta\psi$ by electrogenic K^+ efflux. The net reaction is then electroneutral exchange of K^+ for the ions (mostly H^+) constituting J_{in} . Thus K^+ efflux tends to maintain $\Delta\psi$ and the rate of K^+ efflux is a measure of J_{in} .

For this method to work, K^+ efflux must be the only significant pathway of charge efflux following myxothiazol addition. To ensure that this was so, valinomycin was added in excess. Halving the amount had no effect on the rate of K^+ efflux. The method assumes that the main control of the rate of charge influx is $\Delta\psi$ and that the rate of charge influx is the same in the few seconds after inhibition as it was in the steady state immediately before inhibition. This is reasonable as inhibitors were added to block other energy-linked movement of ions and EGTA to prevent Ca^{2+} uptake, and $\Delta\psi$ changed negligibly over the time taken to measure the potassium efflux (measured by K^+ distribution allowing for small volume changes). A criticism of this technique is that there is a delay of about 1.5–2 s after adding the inhibitor before the potassium efflux stabilizes. This is not serious, however, as $\Delta\psi$ changed by a negligible amount in these few seconds. Also, if the inhibitor antimycin was used instead of myxothiazol (antimycin acts more quickly than myxothiazol, the potassium efflux stabilizes in about one second) we obtained the same value for the q^+/O stoichiometry as we did using myxothiazol. Antimycin was not used routinely because, unlike myxothiazol, it does not fully inhibit respiration. This causes uncertainty in the measurement of the q^+/O stoichiometry when J_o is small.

Infusion of KCl showed that the potassium-sensitive electrode was kinetically competent to measure potassium efflux rates in

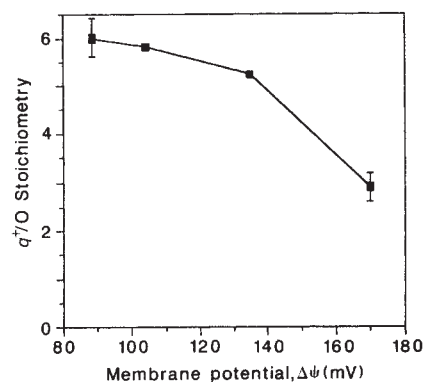


Fig. 3 The q^+/O stoichiometry plotted against membrane potential. The results from Fig. 2 are replotted to show the q^+/O stoichiometry against $\Delta\psi$.

the range encountered here. Addition of a mixture of the uncoupler carbonyl cyanide *p*-trifluoromethoxyphenylhydrazone (FCCP) (0.3 μM) and myxothiazol (2 μM), instead of myxothiazol alone, caused a rate of K^+ efflux about seven times greater than that recorded in Fig. 1, indicating that valinomycin-mediated K^+ transport across the mitochondrial membrane was not limiting the rate of K^+ efflux. If we used a quarter or double the amount of mitochondria and valinomycin used in Fig. 1 the result was unchanged. Thus the potential rate of K^+ transport by the valinomycin was much greater than the net rate of K^+ movement and, although the K^+ concentrations were changing after adding myxothiazol, the inward and outward rates would have been sufficiently large compared to the net rate such that the K^+ and Rb^+ would have been in a state of quasi-equilibrium and the membrane potential could be calculated from the Nernst equation. Other controls showed that myxothiazol does not uncouple mitochondria. We conclude that the method correctly measures the q^+/O ratio.

The experiment shown in Fig. 1 was repeated using a range of different malonate concentrations to decrease J_o and lower Δp (malonate is a competitive inhibitor of succinate dehydrogenase). J_o , the q^+/O stoichiometry and $\Delta\psi$ were measured and are shown in Figs 2 and 3. Under these conditions ΔpH was negligible, hence $\Delta\psi$ was approximately equal to Δp . The well-known nonlinearity of the relationship between $\Delta\psi$ and J_o is shown in Fig. 2. The important new finding, shown in Fig. 2, was that the q^+/O ratio rose from about 3 to about 6 as the steady-state respiration rate and $\Delta\psi$ were progressively reduced with malonate. Figure 3 shows the relationship between q^+/O and $\Delta\psi$; there was a gradual increase in q^+/O as $\Delta\psi$ dropped from 170 mV to 100 mV. The q^+/O stoichiometry of 6 found at the lowest values of $\Delta\psi$ is in agreement with results obtained by most other methods which measure the q^+/O stoichiometry at low values of $\Delta\psi$ (refs 1–3). Results similar to these were obtained if the inhibitor myxothiazol was used instead of malonate to set different steady states.

The change in stoichiometry (or 'slip') we have reported here could, in principle, occur in the cytochrome bc_1 complex, cytochrome oxidase or in both. A lowering of stoichiometry of the cytochrome bc_1 complex at high ΔpH has been reported⁹, but other results¹⁴ suggest that the stoichiometry of the cytochrome bc_1 complex is unchanged at high values of Δp . The results we present disagree with an earlier paper from our laboratory¹⁵ which showed that the q^+/O stoichiometry was invariant over a range of $\Delta\psi$ values from 170 mV to 70 mV. We now believe that $\Delta\psi$ was overestimated by 30–40 mV in that study due to the approximate calibration of the safranine absorbance by K^+ diffusion potentials. The internal K^+ was assumed to be 120 mM and the contribution of K^+ from the mitochondria to the external K^+ was ignored. Both of these assumptions are incorrect and

lead to an overestimation of $\Delta\psi$. Cytochrome oxidase can be induced to slip by chemical modification¹⁶ which may be related to the drop in q^+/O ratio that we report here.

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Formation of disulphide-linked $\mu_2\omega_2$ tetramers in pre-B cells by the 18K ω -immunoglobulin light chain

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Pre-B cells are precursors of B lymphocytes that contain intracellular heavy-chain protein (μ) and are either yet to rearrange their light-chain genes or are in the process of doing so¹⁻⁴. These cells have traditionally been considered to contain intracellular μ -chain with no associated light chain. We demonstrate here that pre-B lymphoid lines synthesize a protein of relative molecular mass (M_r) 18,000 (18K), which we term ω , which forms disulphide-linked $\mu_2\omega_2$ tetramers. This protein could be immunoprecipitated with μ -chain from pre-B lines, but not from T-cell and fibroblast lines that express transfected μ -genes, nor from a pre-B line that synthesizes a $D\mu$ -protein (which lacks a V domain). We view the ω -chain as being a pre-B specific surrogate light chain that may be essential for the important regulatory function that the μ -protein is believed to have at this stage of differentiation.

Intracellular immunoglobulin, immunoprecipitated from a range of cell lines that had been metabolically labelled with ³⁵S-methionine, was analysed after reduction by electrophoresis through 12.5% polyacrylamide-SDS gels (Fig. 1a and b). The origins of these cell lines are given in Table 1. In all pre-B cell lines containing an intact heavy chain protein, an 18K protein (ω) was found coprecipitated with μ -protein (Fig. 1a, lane 2; b, lanes 2 and 3; c, lane 2). A fibroblast cell line expressing a transfected complementary DNA encoding μ_m (the membrane form of the μ -protein) was not found to contain a similar associated protein (Fig. 1a, lane 3 and b, lane 1). Certain pre-B lines, which have partially rearranged their immunoglobulin heavy chain genes, synthesize a truncated $D\mu$ protein from a $D-J_H$ transcript⁵. One such line is 300-19, an Abelson pre-B cell. The $D\mu$ protein that was immunoprecipitated from this line was not associated with the ω -protein (Fig. 1a, lane 1)

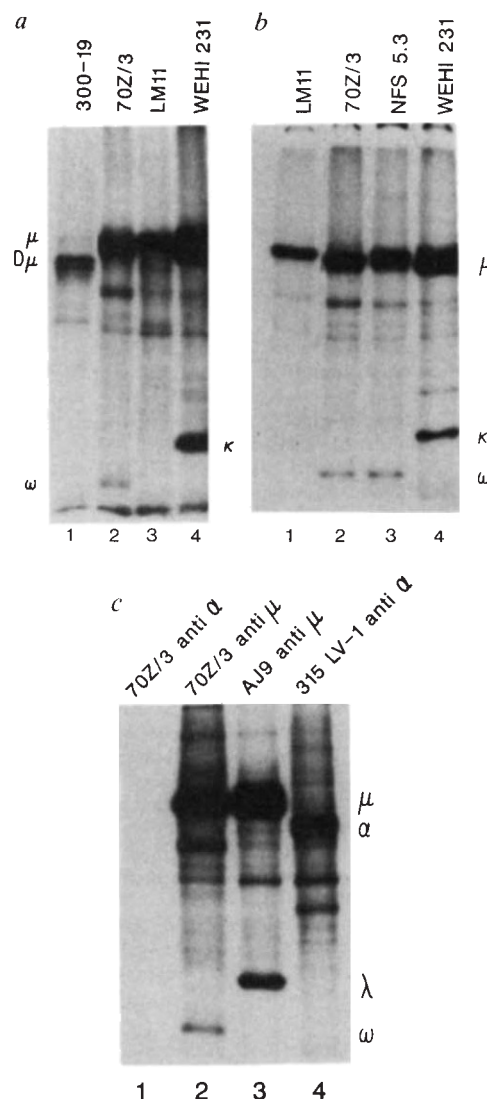


Fig. 1 Immunoprecipitation of μ and α from metabolically labelled cell lines followed by analysis on 12.5% polyacrylamide-SDS gels. Pre-B cell lines included 70Z/3 (ref. 24) (a, lane 2; b, lane 2 and c, lanes 1 (anti- α) and 2 (anti- μ)); NFS 5.3 (ref. 25) (b, lane 3) and 300-19 (ref. 5), a line which expresses the $D\mu$ protein (a, lane 1). WEHI 231 (ref. 26) and AJ9 (ref. 27) are B-cell lines representing the virgin B stage (a, lane 4; b, lane 4 and c, lane 3). 315 LV-1 (c, lane 4) is a light-chain-minus variant of the MOPC 315 α -myeloma cell line²⁸. LM11 (a, lane 3 and b, lane 1) is a derivative of mouse L cells expressing a μ_m cDNA (data not shown). **Methods.** Cells were metabolically labelled with ³⁵S-methionine for 150 min, lysed, and α - or μ -protein was immunoprecipitated essentially as in ref. 1. Samples were reduced and 12.5% polyacrylamide-SDS gel electrophoresis was performed as described before¹.

suggesting that a μ -chain V domain may be necessary for μ - ω association. We did not find any coprecipitated ω -chain in two B-cell lines, WEHI 231 (Fig. 1a, lane 4 and b, lane 4) and AJ9 (Fig. 1c, lane 3); it is conceivable that these lines actually synthesize ω -protein, but because they also express an excess of κ or λ light-chain protein, the ω -chain which might be present would not be complexed to a μ -chain. But in MOPC 315 LV-1, a chain-loss variant of the myeloma cell line MOPC 315 which does not express light chain, precipitation with antibody against α -heavy-chain did not coprecipitate the ω -chain (Fig. 1c, lane 4), suggesting that it may be expressed only at the pre-B stage. Also, ω -protein was not precipitated from pre-B lines containing μ -chains when non-immune serum or an antiserum directed

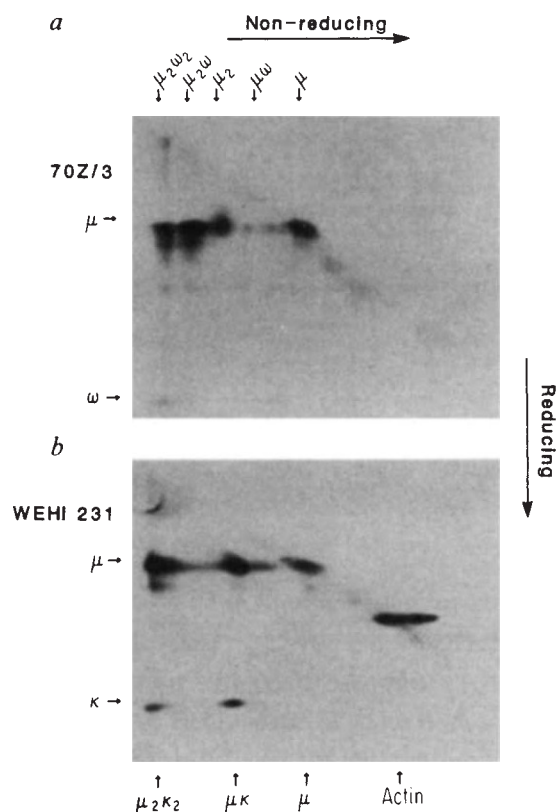


Fig. 2 Analysis of immunoprecipitates from pre-B and B cells, respectively, by two-dimensional non-reducing-reducing polyacrylamide-SDS gel analysis. *a*, Analysis of 70Z/3 immunoprecipitate (pre-B); *b*, analysis of WEHI 231 immunoprecipitate (B-cell).

Methods. Metabolic labelling and immunoprecipitation were as described in Fig. 1. Immunoprecipitates were run on individual 10% polyacrylamide-SDS tube gels without prior reduction of samples. Individual tube gels were immersed in 0.0625 M Tris pH 6.8 containing 5% (v/v) 2-mercaptoethanol for 2 h. Tube gels were placed horizontally on 12.5% polyacrylamide-SDS slab gels, sealed with 1% agarose and electrophoresed in the second dimension.

against the α -isotype was used for immunoprecipitation (Fig. 1c, lane 1). The range of cell lines examined for ω -chain is shown in Table 1.

To determine whether the ω -protein was covalently associated with μ -protein through a disulphide bond (in the manner of conventional heavy-light chain association), we examined immunoprecipitates from the pre-B cell line 70Z/3 and from the B-cell line, WEHI 231, using a two-dimensional polyacrylamide-SDS gel system (Fig. 2). Immunoprecipitated samples were boiled in SDS in the absence of a reducing agent and were

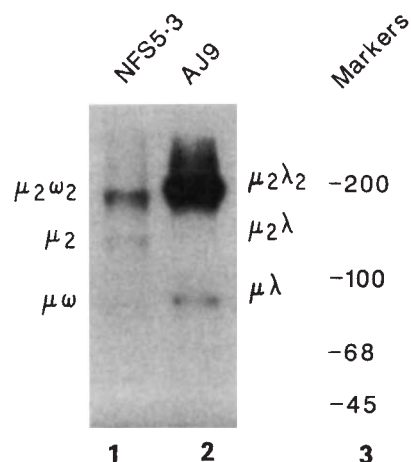


Fig. 3 Immunoprecipitation of surface labelled μ -protein from cell-surface-iodinated NFS 5.3 and AJ9 cell lines followed by analysis of non-reduced samples on a 5–15% gradient polyacrylamide-SDS gel. Lane 1, anti- μ immunoprecipitate from cell-surface-iodinated NFS 5.3 cells; lane 2, anti- μ immunoprecipitate from cell-surface-iodinated AJ9 cells; lane 3, Non-reduced size markers: relative molecular mass in thousands.

Methods. Cells were surface-iodinated by the lactoperoxidase-glucose oxidase method²⁹ and lysates were prepared and immunoprecipitated as described above.

separated on 10% polyacrylamide-SDS tube gels which constituted the first dimension. The first-dimensional gel was then reduced in 2-mercaptoethanol to cleave intra- and interchain disulphide bridges and the gel was placed horizontally over a 12.5% polyacrylamide-SDS slab gel which constituted the second dimension. Oligomeric proteins not linked by sulphhydryl bonds would be expected to migrate, along with non-oligomeric proteins, identically in both dimensions and would be recovered along a diagonal. Oligomers linked by disulphide bonds would appear off the diagonal, allowing their individual subunits to be identified.

In Fig. 2*b*, off-diagonal μ - and κ -bands corresponding to the $\mu\kappa$ and $\mu_2\kappa_2$ species that would be expected in B cells are identifiable. In Fig. 2*a*, off-diagonal spots containing both μ -chain and ω -chain are evident in 70Z/3 a pre-B cell line. We interpret the various spots from their mobilities relative to the species in WEHI 231 cells as μ , $\mu\omega$, μ_2 , $\mu_2\omega$, $\mu_2\omega_2$ species. It is evident that some of the μ -chain in pre-B cells is complexed covalently to ω -chain.

In a few pre-B cell lines which have not rearranged their light chain genes, μ_m has been reported to be on the cell surface in the absence of light chain protein. One such line is NFS 5.3 (ref. 25), in which we had detected intracellular ω -protein as described above (Fig. 1*b*, lane 3). Cell-surface iodination of this cell line and of a B-cell line (AJ9) followed by immunoprecipitation with antibody against μ -protein and analysis of non-

Table 1 Cell lines analysed for ω -protein

Cell type	Name	Mouse strain	Ref.	H chain	Conventional L chain	ω -protein
Non-lymphoid	LM11	C3H/AN		μ	—	—
T cell	BW μ 13	AKR	*	μ	—	—
Pre-B	54.3	C57BL/6	6	μ	—	+
	70Z/3	BALB/c	24	μ	—	+
	NFS 5.3	NFS	25	μ	—	+
	300-19	BALB/c	5	D μ	—	—
B cell	WEHI 231	BALB/cxN3B	26	μ	κ	—
	AJ9	A/J	27	μ	λ	—
Plasma cell	315 LV-1	BALB/c	28	α	—	—

*Y. Bergman and D.B., unpublished data.

reduced samples on a 5–15% polyacrylamide-SDS gradient gel revealed that the major labelled species on the surface of NFS 5.3 is a $\mu_2\omega_2$ tetramer (Fig. 3, lanes 1 and 2). Not only does μ -chain form disulphide-linked $\mu_2\omega_2$ tetramers in pre-B cells, but in the few pre-B cell lines in which μ -protein is transported to the cell surface in the absence of conventional κ - or λ -light-chains, it is transported to the cell surface as a $\mu_2\omega_2$ tetramer. This strongly suggests that the ω -chain is a pre-B specific 'surrogate' light chain.

Considerable evidence, both from cell lines that differentiate in culture as well as from experiments that involve the expression of exogenous immunoglobulin genes in transgenic mice^{6–9}, supports an intracellular function for the μ -heavy-chain protein in the feedback regulation of B-cell differentiation. Recent evidence⁹ suggests that μ_m (the membrane form of the μ heavy chain) alone may be sufficient to mediate the feedback regulation process. A 78K heavy chain binding protein (Bip) was initially described in a heavy-chain-only myeloma¹⁰, and it was considered that it might help μ -protein to mediate regulation^{11,12}. This protein has been identified as the ubiquitously expressed glucose regulated protein, grp78, which binds transiently and non-covalently to a number of secretory proteins in a range of cell types^{13,14}. Studies of the Bip/grp78 protein^{15,16} suggest that in heavy-chain-only cells it may be involved in preventing secretion of unassembled secretory immunoglobulin. The grp78 protein was not evident in the analyses described here because it co-migrates with μ -protein. It is probably present, but its ubiquitous occurrence makes it unlikely that it is involved in regulation.

It has been suggested that if the μ -protein were synthesized in large amounts in the absence of light chain it would perhaps precipitate out and be toxic to cells synthesizing it¹⁷. One possible function for ω -protein is to prevent such toxicity in pre-B cells. Although reduced and alkylated heavy chain protein is relatively insoluble *in vitro*, microinjection of heavy-chain messenger RNA into *Xenopus* oocytes has suggested that *in vivo* this protein readily forms soluble and presumably non-toxic heavy chain dimers¹⁸. This result is consistent with our finding that L cells transfected with μ -chain genes can produce the protein in the absence of ω -protein and not be killed. Perhaps Bip may serve this function if it is necessary¹⁹.

In pre-B cells from normal mouse bone marrow, μ_m has been identified as the major form of μ -protein and this protein has a short half-life²⁰. We have observed that in pre-B lines, which synthesize ω -protein, μ_m has a short half-life but in non-lymphoid lines expressing a transfected μ_m gene, the μ -protein is stable and had a long half-life (data not shown). Association of μ_m with ω -protein could be responsible for its pre-B specific fate.

Recently, a pre-B specific gene termed λ_5 was identified in a pre-B minus B subtractive cDNA library. This gene has been cloned and sequenced^{21–23}, but to date no protein corresponding to this gene has been identified. This gene is expressed exclusively in pre-B cells; no λ_5 transcripts have been identified in B cells, T cells, a range of non-lymphoid lines and a host of mouse tissues. The sequence of this gene predicts a 209-amino-acid protein; cleavage of a predicted N-terminal hydrophobic signal sequence would yield a mature protein that is either 173 or 179 amino acids in length. The C-terminal half of this protein resembles the constant domain of a mouse λ light chain. A cysteine is found in position 208 which corresponds to the pre-terminal cysteine in light chain proteins that is involved in covalent heavy-light chain association. This pre-B specific gene is not rearranged during development. The ω -protein may well prove to be the product of the λ_5 gene.

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An octamer oligonucleotide upstream of a TATA motif is sufficient for lymphoid-specific promoter activity

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The octamer sequence ATGCAAT or its inverse complement ATTTCAT is well-conserved in all immunoglobulin gene promoters^{1,2} and has been implicated in promoter function by deletion analysis^{3–7}. Although immunoglobulin promoters are tissue-specific^{3–6,8}, the octamer is also a functional element in non-tissue-specific upstream regions—like those controlling U1 and U2 small nuclear RNA and histone H2B genes—where it is associated with additional canonical elements^{9–12}. Specific interactions occur between the octamer motif and both lymphoid-specific and ubiquitous proteins^{13–18}. By using a synthetic octamer oligonucleotide inserted upstream of the β -globin TATA box we show here that the octamer element by itself is sufficient for directing lymphocyte-specific RNA synthesis when within 70 base pairs of the start site of transcription. We also demonstrate that mutations in any position of the conserved motif interfere with this function.

To examine whether the octamer motif is sufficient to mediate lymphocyte-specific gene expression, we constructed hybrid clones using, as a test gene, β -globin with a downstream simian virus 40 (SV40) enhancer. The promoter region of this gene was truncated upstream of the TATA box, removing the CCAAT-consensus sequence and other conserved sequences of β -globin promoters¹⁹. Instead of these upstream sequences we inserted a synthetic oligonucleotide containing the octamer element in either orientation. Polylinker sequences flanking the octamer contributed, in both orientations, a tenth base pair (bp), the conservation of which had been previously suggested². The resulting constructs contained the wild-type octamer sequence at –54 to –61 relative to the cap-site (Fig. 1a). A control lacking the oligonucleotide was examined in parallel.

Transcriptional activity was determined by quantitative nuclease-S₁ analysis of accumulated RNA²⁰ after transfection of constructs into either NIH 3T3 fibroblasts or the myeloma cell line MPC11. Plasmids with the octamer oligonucleotide in

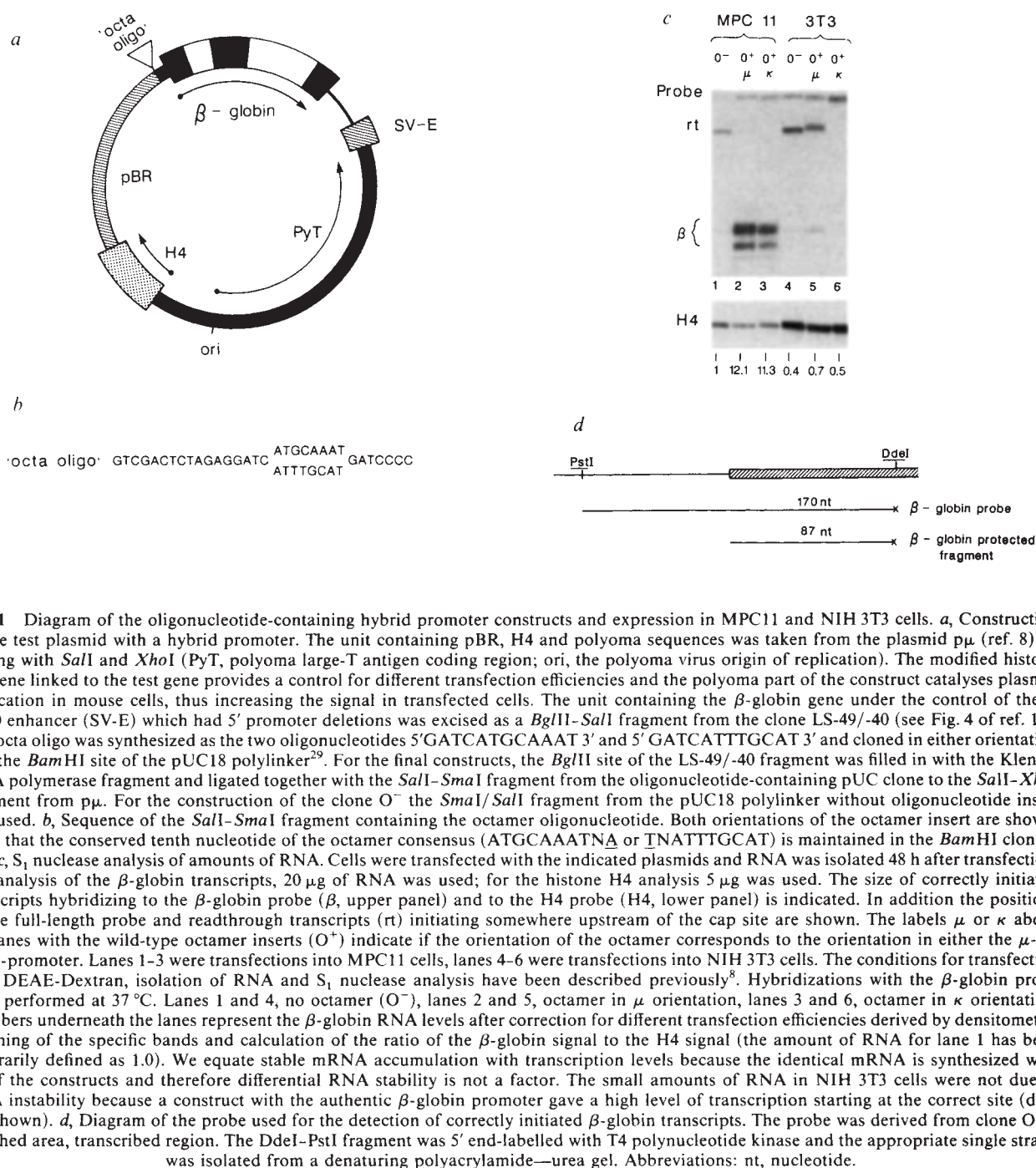


Fig. 1 Diagram of the oligonucleotide-containing hybrid promoter constructs and expression in MPC11 and NIH 3T3 cells. **a**, Construction of the test plasmid with a hybrid promoter. The unit containing pBR, H4 and polyoma sequences was taken from the plasmid p μ (ref. 8) by cutting with *Sall* and *XhoI* (PyT, polyoma large-T antigen coding region; ori, the polyoma virus origin of replication). The modified histone H4 gene linked to the test gene provides a control for different transfection efficiencies and the polyoma part of the construct catalyses plasmid replication in mouse cells, thus increasing the signal in transfected cells. The unit containing the β -globin gene under the control of the 3' SV40 enhancer (SV-E) which had 5' promoter deletions was excised as a *BglII-SalI* fragment from the clone LS-49/-40 (see Fig. 4 of ref. 19). The octa oligo was synthesized as the two oligonucleotides 5'-GATCATGCAAAT 3' and 5'-GATCATTGTCAT 3' and cloned in either orientation into the *BamHI* site of the pUC18 polylinker²⁹. For the final constructs, the *BglII* site of the LS-49/-40 fragment was filled in with the Klenow DNA polymerase fragment and ligated together with the *Sall-SmaI* fragment from the oligonucleotide-containing pUC clone to the *Sall-XhoI* fragment from p μ . For the construction of the clone 0⁻ the *SmaI/SalI* fragment from the pUC18 polylinker without oligonucleotide insert was used. **b**, Sequence of the *Sall-SmaI* fragment containing the octamer oligonucleotide. Both orientations of the octamer insert are shown. Note that the conserved tenth nucleotide of the octamer consensus (ATGCAAATNA or TATTTGTCAT) is maintained in the *BamHI* cloning site. **c**, S₁ nuclease analysis of amounts of RNA. Cells were transfected with the indicated plasmids and RNA was isolated 48 h after transfection. For analysis of the β -globin transcripts, 20 μ g of RNA was used; for the histone H4 analysis 5 μ g was used. The size of correctly initiated transcripts hybridizing to the β -globin probe (β , upper panel) and to the H4 probe (H4, lower panel) is indicated. In addition the positions of the full-length probe and readthrough transcripts (rt) initiating somewhere upstream of the cap site are shown. The labels μ or κ above the lanes with the wild-type octamer inserts (0⁺) indicate if the orientation of the octamer corresponds to the orientation in either the μ - or the κ -promoter. Lanes 1-3 were transfections into MPC11 cells, lanes 4-6 were transfections into NIH 3T3 cells. The conditions for transfection with DEAE-Dextran, isolation of RNA and S₁ nuclease analysis have been described previously⁸. Hybridizations with the β -globin probe were performed at 37 °C. Lanes 1 and 4, no octamer (0⁻), lanes 2 and 5, octamer in μ orientation, lanes 3 and 6, octamer in κ orientation. Numbers underneath the lanes represent the β -globin RNA levels after correction for different transfection efficiencies derived by densitometric scanning of the specific bands and calculation of the ratio of the β -globin signal to the H4 signal (the amount of RNA for lane 1 has been arbitrarily defined as 1.0). We equate stable mRNA accumulation with transcription levels because the identical mRNA is synthesized with all of the constructs and therefore differential RNA stability is not a factor. The small amounts of RNA in NIH 3T3 cells were not due to RNA instability because a construct with the authentic β -globin promoter gave a high level of transcription starting at the correct site (data not shown). **d**, Diagram of the probe used for the detection of correctly initiated β -globin transcripts. The probe was derived from clone 0⁺ κ . Hatched area, transcribed region. The DdeI-PstI fragment was 5' end-labelled with T4 polynucleotide kinase and the appropriate single strand was isolated from a denaturing polyacrylamide-urea gel. Abbreviations: nt, nucleotide.

either orientation exhibited about 11-fold more transcription in MPC11 cells (Fig. 1c, lanes 2 and 3), than a plasmid lacking the octamer (Fig. 1c, lane 1), but no octamer-dependent stimulation was observed in NIH 3T3 cells (Fig. 1c, lanes 4-6). Thus the addition of the octamer motif to a TATA box reconstituted a functional, lymphoid-specific promoter element. In repeated transfections, 11- to 18-fold stimulations were observed in MPC11 cells, comparable to the 20- to 30-fold tissue specificity of the wild-type κ -promoter element⁵. The observed effect was independent of the polyoma sequences; in non-replicating plasmids, insertion of the octamer oligonucleotide upstream of a TATA box and the bacterial chloramphenicol acetyl transferase (CAT) gene (Δ -56 in ref. 21) stimulated transcription 10-fold in S194 myeloma cells, but not in NIH 3T3 cells (Table 1).

The distance between the octamer element and either the

TATA box or the cap site varies amongst different immunoglobulin promoters². To determine whether differences in the spacing between the octamer and the TATA box influence the rate of transcription, we inserted the octamer at various positions with its initial nucleotide between 47 and 70 nucleotides upstream of the cap-site. These constructs move the octamer motif to different sides of the DNA helix relative to the TATA box or the cap-site. After transfection into MPC11 cells, all clones showed comparable levels of transcription starting at the correct site (Fig. 2a, lanes 2-13), but no octamer-dependent stimulation was detected in NIH 3T3 cells (Fig. 2a, lanes 14-25). Although we cannot formally rule out the possibility that all clones have a sub-optimal spacing between octamer and TATA box, we see no strong dependence on the relative positions of the octamer and TATA box. This result is strikingly

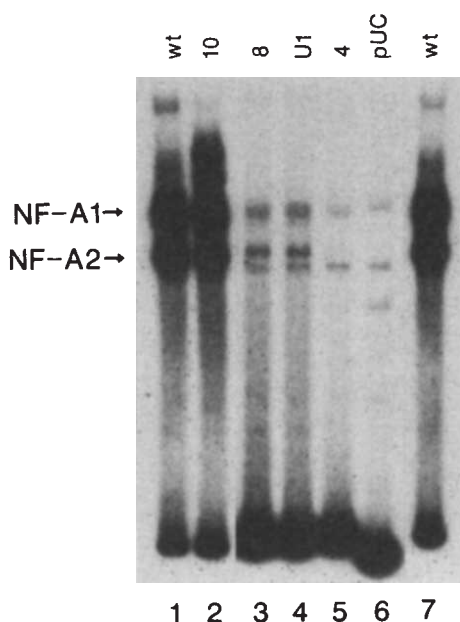


Fig. 4 Binding of nuclear factors to wild-type and mutant octamer motifs. End-labelled *Eco*RI-*Hind*III fragments from the pUC clones containing either a wild-type octamer insert (lanes 1 and 7), the 10th base mutant (lane 2), the 8th base mutant (lane 3), the human U1-like octamer sequence (lane 4), the 4th base mutant (lane 5) or no oligonucleotide insert at all (lane 6) were incubated with a nuclear extract from WEHI 231 cells and analysed by electrophoresis through a low-ionic-strength polyacrylamide gel. NF-A1 and NF-A2 indicate complexes formed by interaction of the DNA with the previously described factors NF-A1 and NF-A2 (see ref. 17), the band at the bottom of the gels represents unbound DNA. Conditions for preparing the nuclear extract as well as gel electrophoresis were as described in ref. 17. All binding reactions contained 1 μ g of the synthetic polymer poly(dI-dC) as nonspecific competitor.

the increase of immunoglobulin messenger RNA levels during B-cell maturation is not due to large increases in the rate of transcription^{8,24,25}.

To determine whether sequence alterations at various positions in the octamer would interfere with its function, we synthesized mutant oligonucleotides containing single transversion mutations at each of the eight positions of the octamer and also in the tenth conserved base². In addition we tested an octamer sequence found to be functional in a human U1-snRNA gene enhancer⁹. All mutations were tested at the same integration site (-54 to -61). A mutation in any of the first 7 bp of the octamer element resulted in baseline transcription levels in both NIH 3T3 and MPC11 cells (Fig. 3b). This result correlates with the lack of variation at any of these positions in the immunoglobulin promoters sequenced to date. The human U1-like octamer sequence likewise was unable to stimulate transcription (Fig. 3c, lanes 9 and 10). Mutations in the eighth and tenth position still allowed reduced lymphoid-specific stimulation of transcription (30–40% and 10–20% respectively, compare in Fig. 3c, lanes 3, 5 and 7). The eight base mutation is found in the promoters of some expressed immunoglobulin promoters^{26,27}, whereas the tenth base mutation has not been reported in any active immunoglobulin gene.

Octamer oligonucleotides with mutations in any of the first seven positions do not efficiently compete for the octamer binding factor(s) in an *in vitro* binding assay¹⁷. The mutants in the eighth and tenth bases, as well as the human U1 analogue, showed specific binding to the two octamer-binding proteins, NF-A1 and NF-A2 (Fig. 4, lanes 2, 3 and 4, compared with lanes 5 and 6), whereas the fourth base mutant did not (Fig. 4,

Table 1 Function of a synthetic octamer promoter in the context of non-replicating constructs

Cell type	O ⁻ -CAT	O ⁺ -CAT	Ratio O ⁺ /O ⁻
S194 myeloma	0.18%	1.8%	10
NIH 3T3 fibroblasts	3.6%	1.95%	0.54

Clones with or without a wild-type octamer insert upstream of the TATA box and the bacterial chloramphenicol acetyl transferase (CAT) gene were transfected into the indicated cell types and CAT enzyme activity was assayed in cell extracts²¹. Values give the percentage of chloramphenicol converted to the acetylated forms. Values for duplicate transfections were averaged and varied <10% from the mean. The higher values for NIH 3T3 are due to higher transfection efficiencies as compared to the myeloma cell lines (data not shown).

lane 5). Binding to the tenth base mutant was comparable to wild type (Fig. 4, lanes 1 and 2). (Additional bands were very probably non-specific protein-DNA interactions; they were also present when the pUC18 polylinker was used as a probe.) Clearly the ability of these new octamer mutants to bind factors *in vitro* did not correlate with their ability to promote transcription. This might indicate that the factors binding *in vitro* are not responsible for the *in vivo* effects, but this conflict could be explained if conditions for nuclear factor binding to its cognate sequence differ *in vivo* and *in vitro*. Alternatively, binding to the wild-type octamer sequence might result in a necessary conformational change that does not occur appropriately with the tenth bp and U1 mutants.

It is evident that given a TATA element, a minimal B-cell specific promoter can be created by a functional octamer sequence. This result suggests, as mentioned previously¹⁷, that the B-cell-specific version of the octamer binding protein (NF-A2) may be able to interact productively with a TATA-binding factor to allow the formation of a transcription complex. The ubiquitous octamer binding factor NF-A1 might only function in conjunction with additional transcription factors. This model explains why the octamer motif is always associated with additional canonical elements in non-tissue-specific promoters^{9–12}. The inability of the octamer sequence found in the human U1 enhancer to stimulate lymphoid-specific transcription also highlights the functional difference between a solitary octamer and one found in association with other functional motifs. It is surprising that a simple sequence motif can act to provide tissue-specific promoter function because most other known promoters utilize multiple sequence motifs²⁸. A possible explanation for the simplicity of the promoter is that for immunoglobulin genes, which rearrange to bring together a promoter-containing variable region with an enhancer-containing constant region, all other functions may be concentrated downstream of the transcriptional start site.

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Millisecond X-ray diffraction and the first electron density map from Laue photographs of a protein crystal

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Attempts to use X-ray crystallography to extract three-dimensional information on transient phenomena in crystals have been hampered primarily by long data collection times. Here we report on the first difference Fourier map obtained from Laue diffraction¹⁻⁸ photographs of a protein crystal, glycogen phosphorylase *b*. Data collection time was 3 s using the high-intensity white X-radiation generated on the wiggler magnet⁹ of the Daresbury Synchrotron Radiation Source (SRS), but data acquisition in the millisecond-submillisecond range is possible. The method presented here uses a simple difference technique and was designed to analyse structural changes relative to a known starting structure. The combination of this approach with cine techniques allows the recording of three-dimensional motion pictures at atomic resolution and opens up new areas in structural biology and chemistry.

Crystallographic studies of changes in structure are restricted by the problem that a structure determined by diffraction methods is averaged over the diffracting volume of the crystal and over the time needed to record the diffraction data. Protein crystal data collection by conventional methods can take days or months, so that biological processes at the molecular level occurring on the picosecond to kilosecond time scale escape observation. This can be partially circumvented by working at low temperatures to freeze out intermediates and to match the time scale of the observation with the time scale of the experiment¹⁰⁻¹². With conventional data collection using monochromatic X-rays, only a small proportion of the lattice planes diffract at any particular orientation of the crystal and the crystal has to be rocked through a small angle (1-2°) to record the full reflections. To bring other planes into the diffracting position, the crystal has to be rotated to a new setting and the procedure repeated with a new film to resolve the diffraction maxima. In the case of glycogen phosphorylase *b*, for example, the monochromatic data set referred to in Figs 2 and 3B required 45 exposures each with a 1° oscillation and exposure time of 500-1,000 s. The total data collection time was 9 h at the synchrotron.

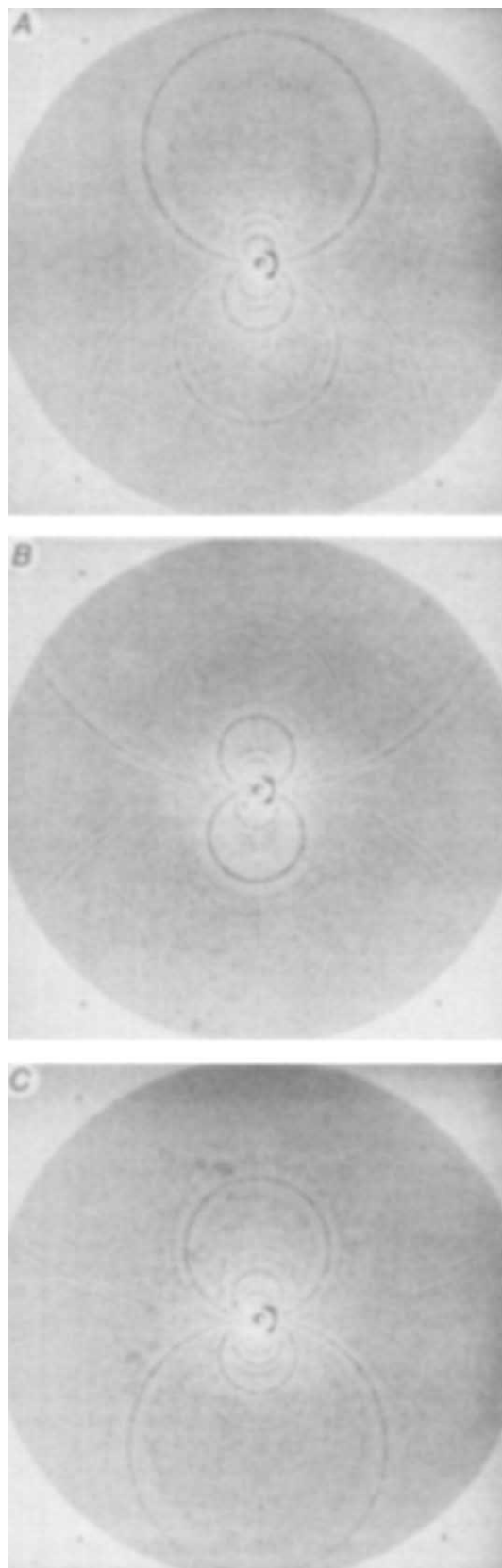


Fig. 1

Fig. 1 (Left). Photographs of the native Laue data set on glycogen phosphorylase *b*. A crystal of phosphorylase *b* (ref. 13) ($P4_32_12$, $a = b = 128.8$ Å, $c = 116.2$ Å, size: $0.3 \times 0.3 \times 2.0$ mm³) was wedged into a slightly tapered quartz capillary of a thermostated flow cell¹⁹. Laue photographs were taken with the crystal rotated to *A*, 11.25°; *B*, 22.5° and *C*, 33.75° from the position with a^* antiparallel to the beam and c^* coinciding with the spindle of an oscillation camera. Crystal to film distance, 133.8 mm; film radius, 59 mm; exposure time, 1 s with a circulating electron beam of 180–150 mA at an energy of 2 GeV; collimator size = 200 µm. The unfocused white beam of station 9.7 of the SRS was used. Effective wavelength range contributing to reflections on the film: 0.2–2.1 Å. Each film pack consisted of 6 Kodak DEF5 films to increase the dynamic range. The top films are shown. Films were scanned at 50 µm raster on a Scandig 3 microdensitometer. The photographs were taken at 20 °C with the crystal surrounded by a buffer of 10 mM N,N-bis-(2-hydroxyethyl)-2-aminoethane-sulphonic acid, 10 mM magnesium acetate, pH 6.74. For photographs with maltoheptose bound to phosphorylase, a solution of 50 mM maltoheptose in the same buffer was pumped through the flow cell (flow rate 0.4 ml per h), assuring a complete exchange of solution around the crystal every second. Total soaking time was 60 min (95–100% saturation). The crystal was surrounded by solution during each exposure.

The extremely high intensity and the broad effective spectrum (0.2–2.1 Å) of the white X-radiation generated on the SRS wiggler magnet⁹ allows the recording of Laue diffraction photographs on the millisecond time scale (data rates of 100,000 reflections per second and higher are possible). With the Laue method, using polychromatic X-rays and a stationary crystal, a large number of planes diffract simultaneously as the Bragg condition is satisfied for each of these planes by at least one wavelength of the spectrum. Many reflections can thus be recorded in a short time with a single exposure. The diffracting position of the lattice planes is determined by their orientation, spacing and the wavelength range applied; the wider the range, the greater the number of planes in diffracting position. Moreover, with crystals of high symmetry, a high proportion of the unique data set may be recorded on a single photograph^{2,7}, providing all the information necessary for structure determination. With crystals of lower symmetry, a few more photographs with different crystal orientations may be required.

Here we present results on tetragonal crystals of glycogen phosphorylase *b* (ref. 13) and show that good-quality electron density maps can be calculated from Laue data. Transient phenomena like structural changes, phase transitions or catalysis may now be investigated by X-ray crystallography, provided that the volume averaging is favourable. With unfavourable volume averaging, that is with mixtures of structural states, it might still be possible to extract structural information on some of the species. This, however, needs further developments, particularly in structure refinement.

The structure of glycogen phosphorylase *b* (EC 2.4.1.1, subunit relative molecular mass (M_r) 97,434 has been solved to 1.9-Å resolution (K. R. Acharya *et al.*, in preparation). The oligosaccharide maltoheptose is a substrate of the enzyme which binds to the glycogen storage site of the protein^{14,15}. In the crystal, the half-saturation binding time is about 8 min (refs 16 and 17). The structure of the bound oligosaccharide is known to 2.5-Å resolution (ref. 18 and P. J. McLaughlin *et al.*, in preparation). In the Laue experiment, the binding of maltoheptose to phosphorylase *b* was monitored. The crystal was kept in a thermostat-controlled flow cell¹⁹ and Laue data sets of three exposures (with six films in a pack) were taken at different angular settings (Fig. 1) before, during, and after the addition of the ligand. The intermediate data set indicates a degree of disorder in the crystal^{7,16,17} and efforts are underway to develop software and methods to extract useful structural information and reveal the time-resolved picture of the binding process. Data sets in the sequence were scaled to the starting (native) Laue data set using an anisotropic temperature factor to compensate for such variations as exposure time, beam decay and radiation damage. Each film in a film pack was individually scaled to its counterpart in the native Laue data set and the measurements were kept unmerged. Because the initial photographs in the

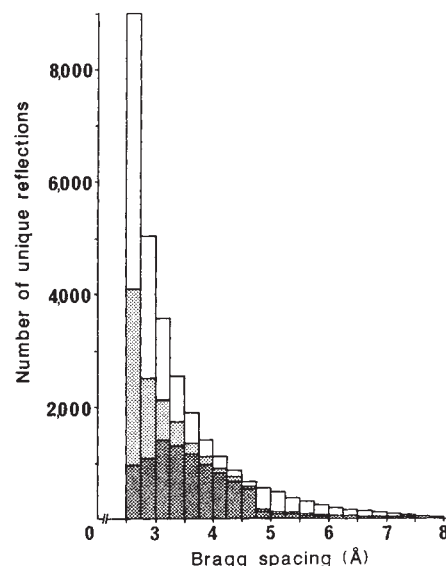


Fig. 2 Distribution of the Laue and monochromatic data as a function of resolution. Open columns, unique portion of a monochromatic data set to 2.5-Å resolution on the maltoheptose-phosphorylase *b* complex (29,689 reflections, P. J. McLaughlin *et al.*, in preparation). Lightly shaded columns, unique portion with 15,529 reflections of the Laue data set on the same complex derived from 121,746 measurement pairs from the native and derivative photographs, where both intensity measurements were positive. Heavily shaded columns, unique portion with the 9,029 reflections of the Laue data set obtained from 76,824 measurement pairs from the native and derivative photographs where both intensity measurements were greater than three standard deviations, and also greater than a quarter of the average intensity of the spots on each of the films.

sequence represent the native protein, and the spectral properties of the X-ray beam were not altered during the experiment, fractional intensity changes on subsequent photographs with the crystal in the same orientation were used to calculate difference Fourier maps with coefficients:

$$\frac{F_{id} - F_{in}}{F_{in}} \times F_{mn}$$

and phases from the monochromatic data^{7,8}. Here, F_{in} is a structure factor amplitude measured on a starting, native photograph; F_{id} is the corresponding measurement after the addition of the ligand; and F_{mn} is the measurement from the reference monochromatic data set. The advantage of this difference technique is that wavelength- and position-dependent corrections are not needed. Significant changes of mass absorption coefficients during the experiment (which might arise from the binding of heavy atoms in the crystal, for example) could necessitate wavelength-dependent absorption corrections for F_{id} , but this was not the case here.

In this experiment, 46.3% of all predicted reflections to 2.4-Å resolution were rejected owing to energy and spatial overlaps (Table 1). Energy overlaps ('multiplets') occur when diffraction maxima for different wavelengths but having the same diffraction angle are superimposed on the film². Although multiplets may be deconvoluted from multiple-film exposures^{6,20}, they represent a small portion of the data (never more than 27.2%, ref. 21) and were not included in the present processing. Spatial overlaps occur because of the very dense nature of protein crystal Laue patterns. After rejections, a unique set of 15,529 reflections with positive intensity measurements ($I > 0$) was obtained (the number of all unique reflections to 2.4 Å resolution is 38,113). For the great majority of the 15,529 unique reflections, the fractional

Table 1 Statistics of Laue measurements

	Angular setting (Fig. 1)			Three photographs together
	11.25°	22.50°	33.75°	
Prediction*:				
Reflections† predicted	49,561	49,474	49,570	148,605
Reflections closer than 0.2 mm (spatial overlaps)	13,269	12,161	12,892	38,322
Spots† to be integrated after rejecting spatial overlaps	30,414	31,197	30,652	92,263
Spots accommodating multiple reflections (energy overlaps)	4,036	4,275	4,149	12,460
Spots with one reflection (singlets)	26,378	26,922	26,503	79,803
Measurement pairs on positive singlets:				
A-films (top)	12,734	13,500	12,209	38,443
B-films	9,203	8,747	8,717	26,667
C-films	7,519	6,992	7,141	21,652
D-films	5,639	5,155	5,349	16,143
E-films	3,973	3,556	3,641	11,170
F-films (bottom)	2,764	2,385	2,520	7,669
Total no. of measurement pairs with each $I > 0$	41,832	40,335	39,577	121,744
Total no. of measurement pairs with both $I > 3 \text{ SD}(I)$ and $I > 0.25 \times I_{\text{average}}$	26,362	27,490	22,972	76,824‡
Unique set from measurement pairs with each $I > 0$	7,123	7,415	7,115	15,529
Fractional isomorphous change in intensity§				
from the Laue data set				0.117
from the corresponding monochromatic data set				0.074
Unique set from measurement pairs with both $I > 3 \text{ SD}(I)$ and $I > 0.25 \times I_{\text{average}}$	4,192	4,501	3,899	9,029‡
Fractional isomorphous change in intensity§				
from the Laue data set				0.043
from the corresponding monochromatic data set				0.068

I , intensity measurement; $\text{SD}(I)$, standard deviation of the intensity measurement.

* Based on parameters given in Fig. 1. The wavelength range (0.2–2.1 Å) and resolution limit (2.4 Å) were deliberately over-estimated to result in an over-prediction. This is a safety measure to prevent predicting multiplets as singlets and accounts for most of the zero intensity measurements.

† A distinction is made between predicted reflections and spots on the film. Harmonic overlaps (multiplets), where the Bragg peaks superimpose exactly, produce a single spot accommodating more than one reflection.

‡ Used for the calculation of the Laue map in Fig. 3.

§ Defined as $\sum |I_1 - I_2| / \sum |I_1 + I_2|$, where I_1 and I_2 are intensity measurements from the derivative and native data sets.

intensity change could be measured at more than one position on a film, on more than one film in a pack, at more than one crystal setting and at more than one wavelength. This gave an average redundancy of about eightfold for the measurements. The positive unique set was further reduced to 9,029 (25% of the possible unique reflections) by selecting reliable measurement pairs only (compare Table 1, Fig. 2). Careful design of both the experiment and data processing software keeps rejections to a minimum (in preparation). Figure 2 shows the distribution of the unique data as a function of resolution. The difference Fourier map showing maltoheptose bound at the glycogen storage site of phosphorylase *b* is shown in Fig. 3A. Figure 3B shows a similar map calculated with a full monochromatic data set to 2.5 Å resolution (29,689 unique reflections). Figure 3C, which represents the real control for the quality of the Laue data, shows a monochromatic map calculated using only those 9,029 reflections common to the monochromatic and Laue data sets (heavily shaded area in Fig. 2). The quality of the Laue map (Fig. 3A) is better than the quality of the equivalent monochromatic map (Fig. 3C). This could be attributed to three major factors: reduced radiation damage (J. Hajdu *et al.*, in preparation); identical conditions for obtaining the native and derivative data sets; and lack of necessity for various corrections in the treatment of the data. To achieve the quality of the full monochromatic map (Fig. 3B), the number of reflections contributing to the Laue map (Fig. 3A) has to be increased. This can be achieved by deconvoluting multiplet spots into component reflections²⁰, by increasing the film size and crystal-to-film distance which reduces the number of spatially overlapping reflections, and by overlap deconvoluting software. Planned new experiments will benefit from each of these techniques.

It is possible to extract structural information from Laue data

without the knowledge of a starting, native structure. The success of this approach does, however, depend on a satisfactory treatment of wavelength-dependent effects such as incident intensity, absorption, Lorentz and polarisation factors, obliquity and detector response^{3,4,6,22,23}. The determination of the so-called 'wavelength normalization curve' to compensate for these effects is a crucial step in the extraction of independent structural information from Laue photographs. None of these factors has to be considered with the difference method outlined above.

Time resolved X-ray studies at the SRS may be carried out on systems with intermediate half-lives ranging from hours (using monochromatic data)^{16,17}, through a few seconds (Laue data), down to picoseconds (stroboscopic experiments using the time structure of the storage ring). These methods have great potential in the three-dimensional study of a variety of

Fig. 3 (Opposite). Difference Fourier maps showing bound maltoheptose in phosphorylase *b* crystals. A, Laue map; B, C, monochromatic map. A, Laue map calculated with a unique set of 9,029 reflections (compare Fig. 2). B, Monochromatic map to 2.5-Å resolution calculated with a unique set of 29,689 reflections. C, Monochromatic map calculated with the 9,029 reflections common to both the monochromatic and the Laue data sets. A positive contour is displayed at half-maximal peak height in all 3 maps. Connectivity did not improve by lowering the contour level in c. The only effect of this was an increase in noise. Side chains of the protein are displayed as they appear in the unliganded native structure. Only four of the seven sugar units can be localized. This is due to the increased mobility of the sugar units at each end of the maltoheptose molecule (these residues protrude into the solvent). The three central sites have the highest occupancies in the full monochromatic map (B) and in the Laue map (A). Movements of Glu 433, Lys 437 and Gln 408 produce the two extra density lobes on either side of the oligosaccharide in B and can clearly be seen in A (and C).

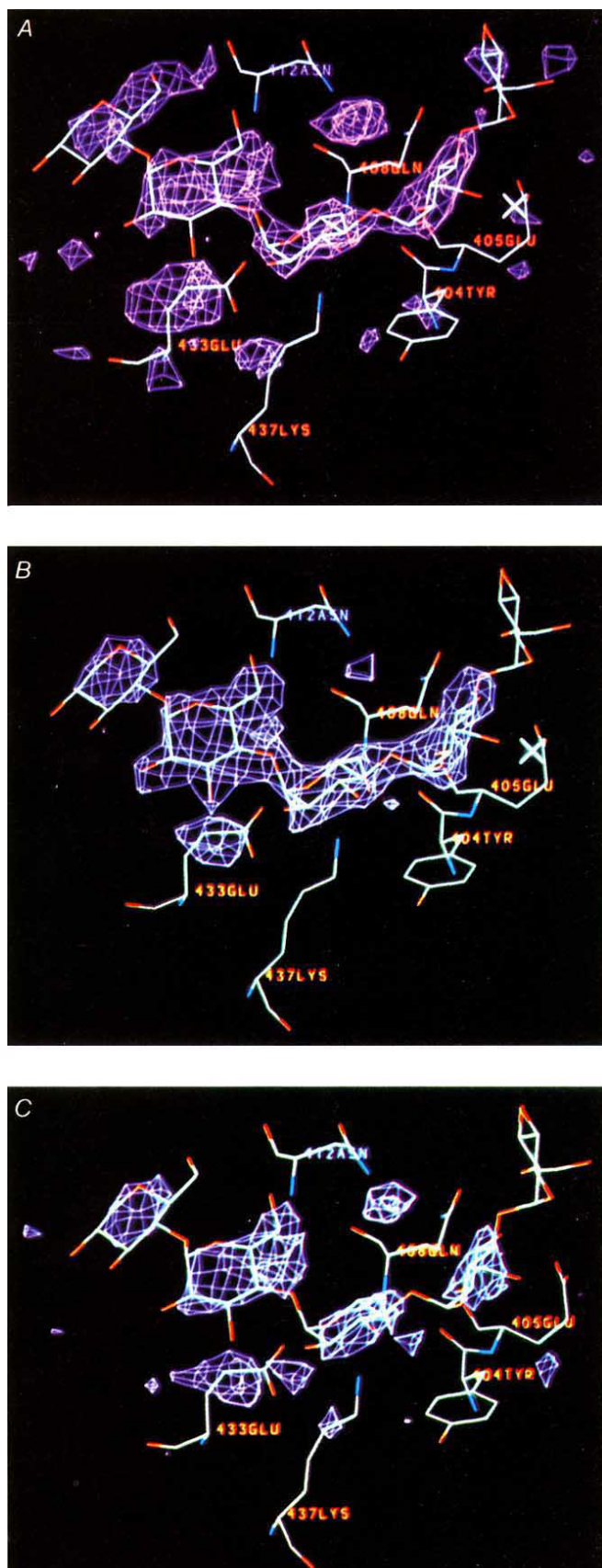


Fig. 3

phenomena, not only in protein crystallography but also in other areas of solid state physics.

We wish to dedicate this paper to the memory of P.A.M. and M.E., killed in a mountaineering accident on 7 March 1987.

Special thanks are due to members of the Protein Crystallography Project Team and the staff of the Daresbury Laboratory for facilities and service at the storage ring. We thank C. E. Jackson for help in scanning the films and Drs D. I. Stuart, K. R. Acharya and R. C. Liddington who were involved in the initial Laue experiments. Thanks also to K. R. Acharya and D. I. Stuart for providing the phases of the refined phosphorylase *b* structure and to Dr P. J. McLaughlin for permission to use unpublished data. This work was supported by the Science and Engineering Research Council and the Medical Research Council, U.K.

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Erratum

Observation of possible superconductivity at 230 K

C. Y. Huang, L. J. Dries, P. H. Hor, R. L. Meng, C. W. Chu & R. B. Frankel

Nature 328, 403-404 (1987).

THE personal communication reported in the first paragraph was from M. L. Cohen, and not M. H. Cohen as published.

Corrigendum

The climate attractor over short timescales

C. Essex, T. Lookman & M. A. H. Nerenberg

Nature 326, 64-66 (1987).

IN this paper equation (2) should be corrected to read $C(r) \sim r^\nu$.

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Laser microprobe mass spectrometry

S. Singh

Minimal sample preparation and the ability to analyse minute quantities of sample are some of the advantages of laser-based spectroscopy.

THE development of new, sophisticated metallic alloys and organic compounds has led to a revolution in the materials world. New alloys, compounds and composite materials have been developed to meet a continual demand for superior mechanical, electrical, magnetic or thermal performances.

The property of a material is dependent upon its elemental makeup and the spatial distribution of the elements within the material. For example, the mechanical strength of an alloy of 99 per cent iron and 1 per cent carbon cannot be predicted unless the distribution of the carbon in the iron matrix is known. Similarly, addition of impurities at the level of only a few parts per million will alter the electrical conductivity of silicon dramatically. However, to predict this change, we need to know not only what the impurity is, but how it is distributed throughout the silicon.

To solve these problems, there is now a demand for analytical instruments that can provide rapid chemical analysis from a small amount of sample with minimal sample preparation. A relatively recent development in such instruments has been brought about by the availability of high-powered lasers. These can be used to vapourize and ionize a small volume of material for subsequent mass analysis.

Instrumentation

Laser microprobe mass spectrometry uses a short pulse from a Q-switched laser to evaporate and ionize a small volume of the material under study. Ions extracted from the resulting microplasma are analysed by time-of-flight mass spectrometry.

In the laser ionization mass analyser from Cambridge Mass Spectrometry Ltd, the sample is viewed through a high-resolution optical microscope system which also serves to focus the laser pulse onto the sample surface. The specific area for analysis is identified and defined by the focused spot of an auxiliary continuous-wave He:Ne laser. Sample viewing, laser irradiation and ion extraction are all performed normal to the sample surface. The laser pulse is provided by a Q-switched Nd:YAG laser, frequency-doubled to give a maximum pulse energy of 150 mJ and 6–7 ns pulse length at a wavelength of 532 nm, or frequency-quadrupled to give up to 80 mJ pulse energy with a 4–5 ns pulse length at 266 nm wavelength. The power density at the sample surface is variable up to 10^{11} W cm⁻².

The folded drift tube of the time-of-

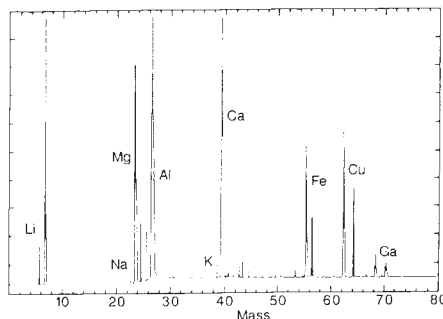


Fig. 1 Positive ion spectrum from the intracellular region of aluminium-lithium alloy.

flight mass spectrometer incorporates an electrostatic reflecting element¹ which provides a second-order time correction for the relatively large spread in the initial energies. Ions are detected by a venetian blind electron multiplier from which the amplified time-spectrum of the arriving ions is acquired by a fast transient recorder. The typical time required for the acquisition of a mass spectrum is approximately 100 microseconds.

Performance

The spatial resolution of the technique is related to the dimensions of the laser-induced crater. This is usually less than 1–3 μ m in diameter and approximately tenths of a micrometre in depth, at a typical laser power density of 10^{10} W cm⁻². These crater characteristics depend strongly on the sample material and the laser power density. The depth removed per pulse has varied from 0.1 μ m for a silicon nitride layer on a microcircuit (R.W. Odom, Charles Evans and Associates, Redwood City, California, personal communication) to approximately 20 μ m for PVC.

The overall mass resolution is on the order of $1,000 M \Delta M^{-1}$, and the time-of-flight technique has the ability to detect quantitatively all ion species resulting from a single laser pulse. Both positive and negative ion mass spectra can be obtained by merely switching voltage polarities. The technique can identify all elements in the periodic table through uranium, and has the ability to discriminate between isotopes. It can also detect molecular ions, yielding additional analytical information.

The relative sensitivity of the technique to different elements appears to lie within a factor of ten at most, and is approximately two under optimum operating conditions. It is more uniform at a wavelength of 266 nm than at 532 nm, and is

near unity for elements of similar ionization potential. The detection limit for trace elements is approximately 10 p.p.m. for a single laser pulse. However, approximately 1 p.p.m. has been achieved for boron in silicon². It may be noted that 1 p.p.m. in an analysed depth of approximately 0.5 μ m corresponds to a few hundredths of a surface monolayer.

Laser probe microanalysis is applicable to a very wide range of materials including not only conductors but semiconductors, glasses and ceramics, polymers and composites, coatings and powders, and organic and biological samples. The range of materials and analytical problems to which laser probe microanalysis may be applied appears to be very wide and has certainly not yet been fully explored. Some early applications of this technique, and further details on the instrument and its performance, may be found in refs 3–5.

The technique is useful in the detection of both organic and inorganic species. Figure 1 is a spectrum derived from aluminium-lithium powder. This particular alloy is currently being used in the development of high strength, lightweight materials. The powder, produced by high pressure gas atomization, solidifies with a cellular structure of approximately 3 μ m. After accurate positioning, the technique has identified a high impurity concentration of typical segregants; copper, gallium, magnesium and iron in the micron intercellular regions, along with some partitioning of lithium on the cell walls. The intracellular regions were shown to be free of impurities.

Figure 2 demonstrates the feasibility of viewing and analysing submicron organic particulates on a semiconductor wafer. Laser microprobe mass spectrometry has been used to analyse 0.5 μ m diameter polystyrene balls mounted onto a silicon wafer. The single-shot positive ion spectra obtained shows ion species at $MZ^{-1} = 77$ and 91, that can be assigned to the cyclic

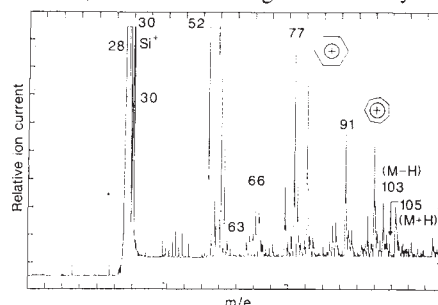


Fig. 2 Positive ion spectrum from 0.5 μ m polystyrene balls mounted on a silicon wafer.

ions $C_6H_6^+$ and $C_7H_7^+$ (tropylium), respectively. Other recognizable peaks due to the molecular structure of polystyrene are $M Z^{-1} = 103$ (M-H) and to a lesser extent $M Z^{-1} = 105$ (M+H), where M refers to the molecular ion in the equivalent monomer.

These are only two applications of laser microprobe mass spectrometry. Other work using this technique includes the observation of beryllium in lung tissue, analysis of explosives, contamination microanalysis of integrated circuits, and the investigation of polymers. □

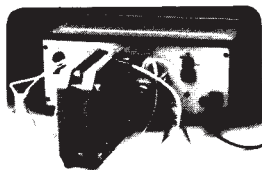
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Reader Service No. 10

Outfitting for analysis

From an accessory for differential weighing to an inductively coupled plasma mass spectrometer, this week's feature offers solutions to analytical problems, large and small.

A JOINT venture between Perkin-Elmer and the SCIEX Division of MDS Health Group Ltd was the genesis of the ELAN **inductively-coupled plasma mass spectrometer** (Reader Service No. 101). The £178,800 (UK) ELAN is designed for multielement determination of elemental species at low concentrations, and is complementary to both ICP emission spectroscopy and graphite furnace AA. The ELAN consists of a plasma source, spectrometer, detection system and computer/controller. Rather than the optical dispersion used in an ion emission instrument, the ELAN uses a spectrometer that disperses ions according to their mass. Special features include multielement isotope dilution analysis, isotope ratio determination and negative ion capability.

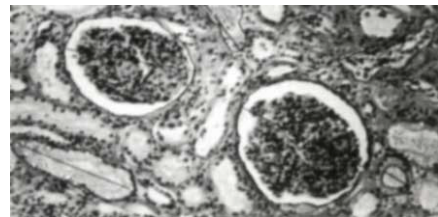
The Evapo-Mix multiple **sample concentrator** from Haake Buchler concentrates ten samples simultaneously from a variety of test tube or centrifuge tube types (Reader Service No. 102). Variable speed mixing with a 750 W immersion heater-thermoregulator assures that the bath has uniform temperature. Bumping is minimized by exposing a large solvent surface area in the sample tubes to heat and vacuum to increase the evaporation rate. The vacuum manifold is available in glass or stainless steel, and has a central water-cooled tube to condense solvent vapours. The temperature in the \$1,995 (US) instrument's bath is maintained to be constant to within $\pm 0.2^\circ\text{C}$.

For the price of a simple integrator — about \$1,995 (US) — the Baseline **chromatography workstation** from Dynamic Solutions, a division of Millipore, can be added to an IBM microcomputer or compatible (Reader Service No. 103). Standard features include the automation of up to 100 sample analyses, batch reanalysis of chromatograms from disk, visual display of linear and nonlinear calibration curves, baseline adjustments and a graphics interface. Options for the basic system include user-customizable plots and reports, a built-in results data base manager, HPLC pump control, valve switching, and advanced analysis routines.

From Dynatech Laboratories comes a computer-controlled **sample preparation system**, the SPD 3000 liquid handling system (Reader Service No. 104). The \$27,000 (US) SPD 3000 uses a tracker ball, similar to that found in a video game,

to set up the instrument and allow fast definition of working areas. A variable speed probe control allows fast movement between samples, and slow entry and exit into test chambers for accuracy. A liquid level sensor determines reagent or sample volume automatically. The system takes bottles, beakers, test tubes, cuvettes, microplates, or a combination of all.

Analytical Measuring Systems has announced an addition to its line of video interactive display systems (VIDS) — the VIDS IV (Reader Service No. 105). The £7,000 (UK) VIDS IV is a **semi-interactive image analyser** designed for IBM AT and compatible computers. Inputs can be taken from colour or monochrome cameras, frame stores and electron or



A sharper image from the VIDS IV. optical microscopes. The VIDS IV has a new statistics program which enables tests to be carried out during and after measurements have been made. Data can be channelled directly into the VIDS statistics program without the need to exit or load data into other programs, enabling users to run VIDS IV continuously without switching disks. Optional software packages include programs for 3-dimensional reconstruction and stereology, and spreadsheets for data manipulation.

Mettler's LabPac-M is an accessory for **differential weighing** (Reader Service No. 106). It can be used in conjunction with a Mettler analytical or precision balance for moisture determination in samples and filter analysis, among other applications. Mettler says the LabPac-M can store the weights of up to 20 tare containers and their respective initial weights. After drying, samples can be weighed again to obtain the weight difference in grams or as a per cent of total weight. The LabPac-M comes with a printer and can be interfaced with a microcomputer. □

These notes are compiled by Carol Ezzell from information provided by the manufacturers. To obtain further information, use the reader service card bound inside the journal. Prices quoted are sometimes nominal, and apply only within the country indicated.